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Establishing the Validity of the Polar V800TM Heart Rate Monitor among Adults with Hypertension

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Establishing the Validity of the Polar V800^{TM} Heart Rate Monitor among Adults with Hypertension

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ABBREVATION LIST

AC	R-R Intervals Edited by Automatic Correction
AV	Atrioventricular Node
ANS	Autonomic Nervous System
BMI	Body Mass Index
BPM	Beats Per Minute
CI	Confidence Interval
ECG	Electrocardiogram
ES	Effect Size
HF	High Frequency
HR	Heart Rate
HRV	Heart Rate Variability
HZ	Hertz
ICC	Intra-correlation Coefficient
LF	Low Frequency
LF/HF	Low Frequency to High Frequency Ratio
LoA	Limits of Agreement
MC	Manual Correction
N-N Intervals	Normal R-R Intervals

NU	Normalized Units
pNN50%	Percentage of Successive Normal R-R Intervals > 50 ms
PNS	Parasympathetic Nervous System
RMSSD	Root Mean Square of Successive Differences in R-R Intervals
R-R Interval	Time between two sequential R waves
SA	Sinoatrial Node
SDNN	Standard Deviation of R-R intervals
SD1	Standard Deviation 1
SD2	Standard Deviation 2
SD2/SD1	Standard Deviation 2 to Standard Deviation 1 Ratio
SNS	Sympathetic Nervous System
TBC	R-R Intervals Edited by Threshold-based Correction
TINN	Triangular Interpolation of R-R Intervals
ULF	Ultra Low Frequency
UN	Uncorrected R-R Intervals
VLF	Very Low Frequency
VO_{2max}	Maximum Oxygen Consumption

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CHAPTER 1: INTRODUCTION

In 1895 a breakthrough in signal analysis occurred with the beginning of the ability to measure the electrical signal of the heart through the electrocardiogram (ECG) (Hurst, 1998). This historical achievement revealed that heart rate (HR) is noticeably irregular, which was unlike the common belief at the time (Shaffer et al., 2014). It is well-established today that a healthy heart is not like a metronome producing monotonously regular beats (Shaffer et al., 2014). The irregularity of HR is the result of the dynamic interplay between the sympathetic and parasympathetic divisions of autonomic nervous system (ANS) (Clifford, 2002; Kamath et al., 2012; Shaffer et al., 2014). The interaction between both systems is modulated by regulatory systems of the body including the cardiovascular, respiratory, endocrine, and central nervous systems and chemoreceptors and baroreceptors (Clifford, 2002; Kamath et al., 2012; McCraty, 2015; Shaffer et al., 2014). The sinoatrial (SA) node integrates the inputs from the ANS and the modulations from the aforementioned regulatory systems to adjust HR against the constantly changing internal and external environment for maintaining homeostasis (Maud & Foster, 2006; Shaffer et al., 2014). This adjustment causes an oscillatory pattern in the HR or beat-to-beat fluctuations in the time period between sequential heartbeats, termed *heart rate variability* (HRV) (Maud & Foster, 2006; Shaffer et al., 2014). Since it is not viable to detect SA node action potentials, the fiducial point of heartbeat (i.e., P-wave) on the ECG should ideally be determined for calculating HRV (Clifford et al., 2006; Peltola, 2012). However, the amplitude of P-waves is too low for computer algorithms to identify them correctly (Clifford et al., 2006; Peltola, 2012). Therefore, the R-waves are used to measure HRV due to their distinguishable amplitude. For this reason, HRV can also be called *R-R variability*.

HRV can be analyzed by time, frequency, and non-linear domain measures calculated from the ECG R-R intervals. Time domain measures demonstrate the variance between sequential R-R intervals and are used to quantify the amount of variability during the recording (Maud & Foster, 2006; McCraty, 2015). The most prominent time domain measures include: 1) standard deviation of normal R-R intervals (SDNN); 2) root mean square of successive differences in normal R-R intervals (RMSSD); and 3) percentage of successive normal R-R intervals greater than 50 ms (pNN50%). Time domain measures are easy to calculate but are not useful to quantify the interplay between sympathetic and parasympathetic divisions (Maud & Foster, 2006; McCraty, 2015).

The frequency domain measures are calculated from the power or variance spectral density analysis of the R-R interval time series (Maud & Foster, 2006; McCraty, 2015). This analysis provides information on how the power (i.e., variance of a rhythm) is distributed as a unit of frequency in the hertz (Hz) (Maud & Foster, 2006; McCraty, 2015). Four frequency bands are determined in the power spectral analysis of the R-R interval time series, including: 1) high frequency (HF ms^2, 0.15-0.40 Hz); 2) low frequency (LF ms^2, 0.04-0.15 Hz); 3) very low frequency (VLF ms^2, 0.0033-0.04 Hz); and 4) ultra-low frequency (ULF, $\leq$0.0033). The discussion over the use and representation of LF ms^2 is still ongoing, but mostly the claim is that LF ms^2 is generated by both the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) as well as blood pressure regulation through baroreceptors or by baroreflex modulation only (Heathers, 2014; Pagani et al., 2012; Reyes del Paso et al., 2013). However, overwhelming majority of the researchers agree that HF ms^2 reflects parasympathetic or vagal modulation (Akselrod et al., 1981; Malik, 1996; Heathers, 2014). In addition, HF ms^2 is also known as respiratory band because of the strong linear relationship between vagal control of the heart and

respiratory variation observed in the time difference between two successive R-waves (i.e., respiratory sinus arrythmia) (Shaffer et al., 2014; Shaffer & Ginsberg, 2017). The normalized units of LF and HF and ratio of LF and HF frequency bands (LF/HF) are also commonly reported frequency domain measures. Although its representation has been a matter of debate due to the disagreements regarding LF ms^2 the LF/HF ratio is used as a marker of sympathovagal modulation, the autonomic state resulting from the mutually opposing influences of the sympathetic and parasympathetic activities (Heathers, 2014; Pagani et al., 1984; Pagani et al., 1986) .

Besides traditional time and frequency domain variables, there are also non-linear measures that show the unpredictability of the R-R interval time series caused by the interactions among the complex regulatory systems of HRV (Shaffer et al., 2014; Shaffer & Ginsberg, 2017). The most common non-linear HRV measures consist of: 1) standard deviation 1 (SD1); 2) standard deviation 2 (SD2); and 3) the ratio of SD2 and SD1 (SD2/SD1). These measures of HRV are analyzed using Poincare plot method, providing a graph displaying the correlation between sequential R-R intervals (Shaffer et al., 2014; Shaffer & Ginsberg, 2017). Even though the non-linear measures have become increasingly reported the physiological representations of such measures are still ambiguous (Shaffer et al., 2014; Shaffer & Ginsberg, 2017). Nevertheless, current literature indicates that SD1 represents the baroreflex sensitivity, diastolic blood pressure, and HR minimum and maximum; SD2 represents baroreflex sensitivity; and SD2/SD1 represents autonomic modulation during 5 min of recording (Brennan et al., 2001; Guzik et al., 2007; Shaffer & Ginsberg, 2017).

Low HRV is associated with reduced ability for an organism to deal with the internal and external causes of stress and counter diseases or recover in timely fashion (McCraty, 2015). The clinical significance of HRV was highlighted by Hon & Lee for the first time in 1965. They

observed that low HRV was the first sign of fetal stress while the HR had no change during labor and delivery (Lee & Hon, 1965).

Therefore, HRV has become a popular physiological marker for clinicians and researchers studying clinical populations 1) to determine the early signs of a pathology, the presence of a chronic disease, or health condition and its treatment prognosis (Pumprla et al., 2002); and 2) to evaluate the ability to cope with stress and the effectiveness of an intervention (e.g., exercise, medical procedure) (Mourot et al., 2004; Mourot & Regnard, 2004). Since then, HRV has been associated with various diseases, cardiovascular disease risk factors, and psychological disorders. The current literature indicates that low HRV is associated with diabetic neuropathy (Ewing et al., 1976), myocardial infarction (Pecyna, 2006), sudden cardiac death (Kudaiberdieva et al., 2007), congestive heart failure (Frenneaux, 2004), metabolic disorders (Stuckey et al., 2014), chronic renal failure (Axelrod et al., 1987), fibromyalgia (Kang et al., 2016) , non-cardiac chest pain (Ong et al., 2013), asthma (Kazuma et al., 1997), anxiety (Cohen & Benjamin, 2006), depression (Carney et al., 2001) , and of relevance to this study, hypertension (Schroeder et al., 2003; Terathongkum & Pickler, 2004). In addition, HRV has been a useful measure for coaches in a variety of sport settings since it provides them with an ability to assess and monitor their athletes' cardiorespiratory fitness (De Meersman, 1993), training intensity, type, and time (Vesterinen et al., 2016), training periodization (Kiviniemi et al., 2007), performance (Plews et al., 2013), overtraining (Mourot et al., 2004), and recovery (Mourot & Regnard, 2004).

1.1.Statement of the Problem

Due to the recent technological advances in signal analysis researchers tend to choose HR monitors over the gold standard criterion ECG for measuring HRV because of their ability to record R-R intervals (i.e., heartbeats) with easily available, simple-to-use, affordable, and wireless features.

The apparent ease of accessing HRV via HR monitors has dramatically extended its study in clinical and athletic populations. Indeed, a recent PubMed search (May 22nd, 2019) showed that the number of publications associated with HRV rose from 39 in 1977, a year of the first Polar (Polar Electro Oy) HR monitor to 1785 in 2018. Additionally, the number of studies published during the last 15 years from 2003 to 2018 (n=15,012) was two times higher than those published during the 25 years from 1977 to 2003 (n=6,876). The substantial increase in the number of publications during the last 15 years corresponds to the introduction of new HR monitor technologies such as Polar RS800™, S810™,S810i™, and the latest version of V800™ as well as HRV software packages such as Kubios HRV (ver 1.1). Given the increasing credibility of HR monitors to accurately detect the R-R intervals and the convenience of HRV software packages in analyzing HRV measures the trend in HRV use among researchers seems likely to continue. However, artifacts (i.e., random distortion of usual R-R intervals) with technical (e.g., missed beats) or physiological (e.g., non-sinus beats) origins can impair the ability of HR monitors to detect accurate R-R intervals to such a degree that HRV measures become no longer meaningful which may potentially diminish the usefulness of HRV. Hence, multiple researchers have stressed the necessity of distorted R-R intervals with technical or physiological origins to acquire reliable HRV measures (De Geus et al., 2019; Kumaravel & Santhi, 2010; Mateo & Laguna, 2003; Nabil & Reguig, 2015; Peltola et al., 2008; Salo et al., 2001).

Several lines of evidence suggested that the manual correction (MC) of artifacts with visual identification of the R-R intervals combined with the choice of a proper correction method is the most accurate method for ensuring comparable HRV measures derived from R-R intervals between ECG and Polar HR monitors (De Geus et al., 2019; Nabil & Reguig, 2015; Peltola, 2012). Recently, (Giles et al., 2016) updated the MC methods developed by (Gamelin et al., 2006) by

adding two more corrections for artifacts that were discovered with the current Polar HR monitor V800^{TM} among a healthy population. However, the MC is a laborious process and it requires a certain level of specialty and expertise, which led to the manufacturing of various HRV software packages offering easy-to-use automatic correction options to edit artifacts.

Kubios is the most popular HRV software that is used to automatically correct artifacts cited in about 1000 studies (Kubios HRV [ver. 3.2] User's Guide, 2019). The latest version of the software (Kubios HRV Premium [ver. 3.2]) released in January 2019 provides two options for correcting technical and physiological artifacts, the: 1) automatic correction (AC) and 2) threshold-based correction (TBC) methods. The AC option was not available in the versions before the first release of commercial Kubios HRV Premium (ver. 3.0) in January 2017. The AC uses a time series with differences between sequential R-R intervals to identify artifacts, thereby separating the physiological and technical artifacts from the sinus rhythm (Kubios HRV User's Guide, 2018). While missed beats are edited by adding new R-R intervals, extra beats are corrected by eliminating extra R-R intervals (Kubios HRV [ver. 3.2] User's Guide, 2019). The TBC makes comparisons of each R-R interval against a local R-R interval average (Kubios HRV [ver. 3.2] User's Guide, 2019). If an R-R interval exceeds or falls behind the local R-R interval average more than a selected threshold value, the particular R-R interval is plotted as an artifact for correction (Kubios HRV [ver. 3.2] User's Guide, 2019). Several threshold values are available with this correction method, comprising: 1) very low (0.45 sec); 2) low (0.35 sec); 3) medium (0.25 sec); 4) strong (0.15 sec); 5) very strong (0.05 sec); and 6) custom (a customized threshold in sec) (Kubios HRV [ver. 3.2] User's Guide, 2019). With the TBC method, it is recommended to select the lowest threshold possible to avoid overcorrecting the normal R-R intervals (i.e., N-N intervals) after visually detecting the artifacts as higher thresholds can lead to measurement bias. Both

correction methods of Kubios HRV Premium (ver 3.2) replace the detected artifacts with interpolated R-R intervals using a cubic spline interpolation (Kubios HRV [ver. 3.2] User's Guide, 2019).

The previous versions of Polar including S810™ (Gamelin et al., 2006; Kingsley et al., 2005), S810i™ (Vanderlei et al., 2008), RS800CX™ (Vasconcellos et al., 2015), RS800GX™ (De Rezende Barbosa et al., 2016), and the current version V800™ HR monitors (Caminal et al., 2018); Giles & Draper 2017; Giles et al., 2016) have been validated among healthy individuals, showing good agreement between the corrected R-R intervals as well as HRV measures of the Polar HR monitors and simultaneously recorded 2-lead, 3-lead, 5-lead, and 12-lead ECGs. However, it has been reported that calculation of HRV measures with separate software packages led to incomparable results between ECG and several HR monitors in clinical and healthy populations (Sandercock et al., 2004; Wallén et al., 2012; Weippert et al., 2010). The current version Polar V800™ HR monitor has not been validated in clinical populations, particularly in individuals with hypertension. Moreover, it has been shown that the TBC of the previous version of Kubios HRV (ver 2.2) was not able to correct the certain artifacts properly even though it identified most artifacts correctly, which resulted in large bias between the HRV measures of ECG and Polar V800™ (Giles & Draper, 2017).

HRV is a useful physiological marker for assessing autonomic function. Although the mechanisms causing hypertension are not completely clear one of the potential mechanisms underlying hypertension is a dysregulation/disruption in autonomic function (Schroeder et al., 2003). Therefore, validating a portable, non-invasive, easy-to-use technology such as Polar V800™ that can assess autonomic function in patients with hypertension may help clinicians and researchers better evaluate mechanisms underlying a patient's hypertension and more effectively

target therapeutic options. Moreover, since the clinical populations may have a greater number of artifacts than healthy individuals (Kamath & Fallen, 1995; Peltola et al., 2012), the accurate correction of artifacts for adults with hypertension is even more important to obtain valid HRV measures. This requires researchers to be able to choose a method of correction with the highest editing accuracy. Therefore, establishing the accuracy of the recent version of Kubios HRV Premium (ver 3.2) with its updated TBC and newly added AC methods and recently updated the MC method to correct artifacts from the Polar V800^{TM} HR monitor can assist researchers studying HRV in adults with hypertension to select a method of correction that can produce HRV measures with highest agreeability between the Polar V800^{TM} HR monitor and the gold standard ECG.

1.2.Specific Aims and Hypotheses

Specific Aim 1: To compare the corrected and uncorrected (UN) R-R intervals obtained from Polar V800^{TM} HR monitor to R-R intervals obtained from a 12-lead gold standard ECG among individuals with hypertension.

We **hypothesize:** The Polar V800^{TM} HR monitor will produce corrected R-R intervals consistent and UN R-R intervals inconsistent with a 12-lead ECG among adults with hypertension.

Specific Aim 2: To compare the corrected and UN time, frequency, and non-linear domains of HRV measures derived from corrected and UN R-R intervals obtained from Polar V800^{TM} HR monitor to time, frequency, and non-linear domains of HRV measures derived from R-R intervals obtained from 12-lead ECG among adults with hypertension.

We **hypothesize:** The Polar V800^{TM} HR monitor will produce time, frequency, and non-linear domains of HRV measures derived from corrected R-R intervals consistent and the HRV measures derived from UN R-R intervals inconsistent with time, frequency, and non-linear domains of HRV measures derived from a 12-lead ECG among individuals with hypertension.

Specific Aim 3: To compare the MC and the Kubios HRV Premium (ver.3.2) TBC and AC methods to accurately and reliably correct the artifacts (i.e., distorted R-R intervals) obtained from V800^{TM} HR monitor compared to R-R intervals obtained from 12-lead ECG.

We **hypothesize:** The MC will correct artifacts obtained from V800^{TM} HR monitor with a greater accuracy and reliability than the Kubios Premium (ver.3.2) TBC and AC methods compared to R-R intervals obtained from 12-lead ECG.

CHAPTER 2: REVIEW OF THE LITERATURE

2.1. Overview of Heart Anatomy and Physiology

2.1.1. Heart Anatomy and Circulation

The heart is divided into four chambers bordered by myocardium (i.e., heart muscle). These chambers include the upper left and right atria and lower left and right ventricles. The two atria receive venous blood returning to the heart, whereas the two ventricles pump the blood from the heart to lungs and arteries. Venous blood with a lower content of oxygen flows into right atrium and from there directly into the right ventricle where the pulmonary arteries carry the venous blood to the lungs for replacing oxygen and removing wastes (Kamath et al., 2012; Maud & Foster, 2006; McCraty, 2015).

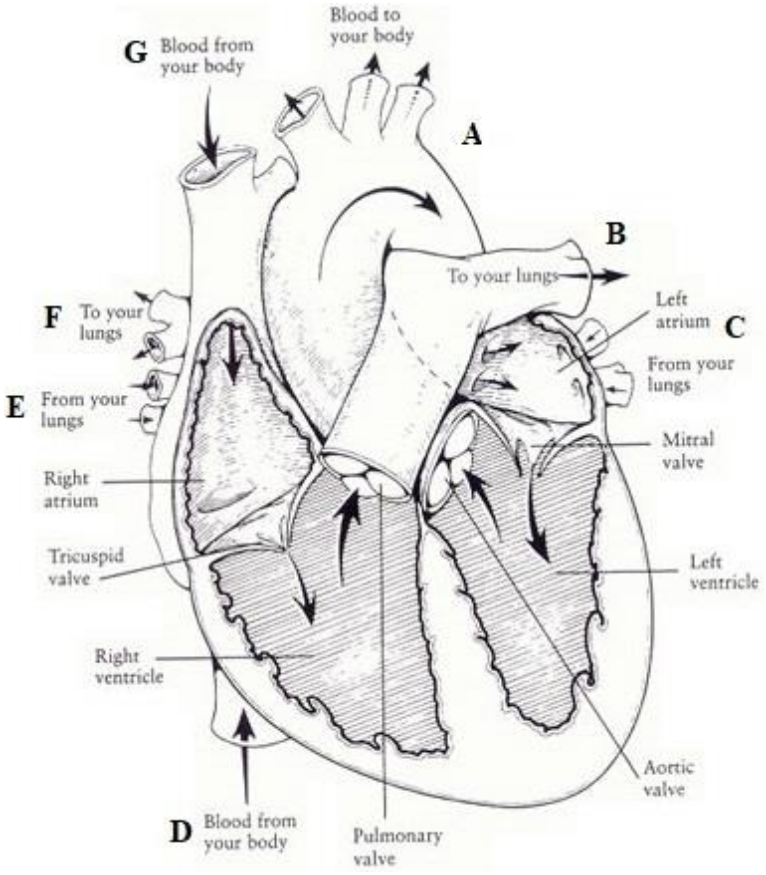


Figure 1. Heart Anatomy and Circulation. A: Aorta; B: left pulmonary artery; C: left pulmonary vein; D: inferior vena cava; E: right pulmonary vein; F: right pulmonary artery; G: superior vena cava. Credit: clipart-library.com

The oxygen-rich blood is moved into the left atrium through pulmonary veins and from there to the left ventricle where the aorta transports the blood to the systemic circulation to meet the oxygen needs of the tissues (Kamath et al., 2012; Maud & Foster, 2006; McCraty, 2015). Interatrial septum ,interventricular septum, mitral, tricuspid, pulmonic, and aortic valves keep blood from flowing back into atria and ventricles (Kamath et al., 2012; Maud & Foster, 2006; McCraty, 2015).

The heart cycle defines the period from the start of one heartbeat to the start of the next. It comprises ventricular contraction, a period where the left ventricle ejects blood from the heart (systole) and ventricular relaxation, a period where the left ventricle relaxes and refills with blood (diastole) (Kamath et al., 2012; Maud & Foster, 2006; McCraty, 2015). Myocardial (relating to the heart muscle) contractions are initiated by a series of electrical impulses, the rate of which determines the HR. The cells that generate the electrical impulses are called pacemakers. two internal pacemakers initiating the heartbeat are the sinoatrial node (SA), atrioventricular node (AV), and Purkinje fibers (Kamath et al., 2012; Maud & Foster, 2006; McCraty, 2015) (Figure 1).

2.1.2. Autonomic Nervous System

The cardiovascular center is located in the medulla oblongata, a long stem-like structure in the brainstem. It regulates the rate at which the heart beats through nervous and endocrine systems. The nervous system comprises central nervous system and peripheral nervous system (Kamath et al., 2012; Maud & Foster, 2006; McCraty, 2015). The main role of peripheral nervous system is to link the central nervous system to the limbs and organs, i.e., transmitting the signals between brain, spinal cord and the rest of the body (Kamath et al., 2012; Maud & Foster, 2006; McCraty, 2015). The peripheral nervous system has two divisions: 1) somatic nervous system (voluntary motor system); and 2) ANS (involuntary motor system). The ANS branches into the SNS (responsible for fight or flight response) and PNS (responsible for rest and digests response)

(Kamath et al., 2012; Maud & Foster, 2006; McCraty, 2015). The PNS response exerts its influence < 1 sec and it is short lasting, but after the onset of the SNS activation, there is a waiting period of around 5 sec ensued by a stable increase in HR for the next 20-30 sec (Shafer et al., 2014). However, HR regulation cannot be interpreted as a linear sum of two opposite effects as elevated parasympathetic activity may not always indicate a decreased sympathetic activity, or vice versa (i.e., increase in PNS can be related to decrease, increase, or no change in SNS) (Heathers, 2014). The PNS reactivation during HR recovery as the SNS stays elevated after a bout of exercise is an example of non-linear interactions between SNS and PNS (Heathers, 2014). Therefore, it is not appropriate to describe the relationship between the SNS and PNS as demonstrated by a seesaw where one side moves up another side moves down.

2.1.3. Electrical Signal of the Heart

The SA node is located in the upper right area of the right atrium. In a healthy heart, the SA node initiates each heartbeat, generating the depolarization (contraction) of the myocardium (Kamath et al., 2012; Shaffer et al., 2014). The intrinsic frequency of SA node is 60-100 beat per minute. If the SA node is injured and unable to regulate the HR, the AV node, the intrinsic frequency of which is 40-60 beats per minute (bpm) can replace the injured SA node as pacemaker (Kamath et al., 2012; Shaffer et al., 2014). In addition, if the pace making ability of AV is compromised then Purkinje fibers, the intrinsic rate of which is 15-40 bpm can regulate the HR (Kamath et al., 2012; Shaffer et al., 2014). Of note, however, Purkinje fibers are triggered as the last resort in the case of failed SA and AV nodes. The electrical stimulus produced by SA node moves through the atria to the AV node in approximately 0.03 sec and triggers AV node discharge (Kamath et al., 2012; Shaffer et al., 2014). Depolarization of the left and right atria of myocardium produce P-wave (atrial systole) of the ECG. The electrical impulse is rapidly propagated through the bundle of His

and Purkinje fibers descending down both sides of the septum (Kamath et al., 2012; Shaffer et al., 2014). As the electrical impulse travels through this region it conducts the electrical impulse over the ventricles approximately 0.2 sec after the occurrence of the P-wave (Kamath et al., 2012; Shaffer et al., 2014). The Purkinje fibers depolarize ventricular myocytes and result in the QRS complex ensued by S-T segment formation (Kamath et al., 2012; Shaffer et al., 2014).

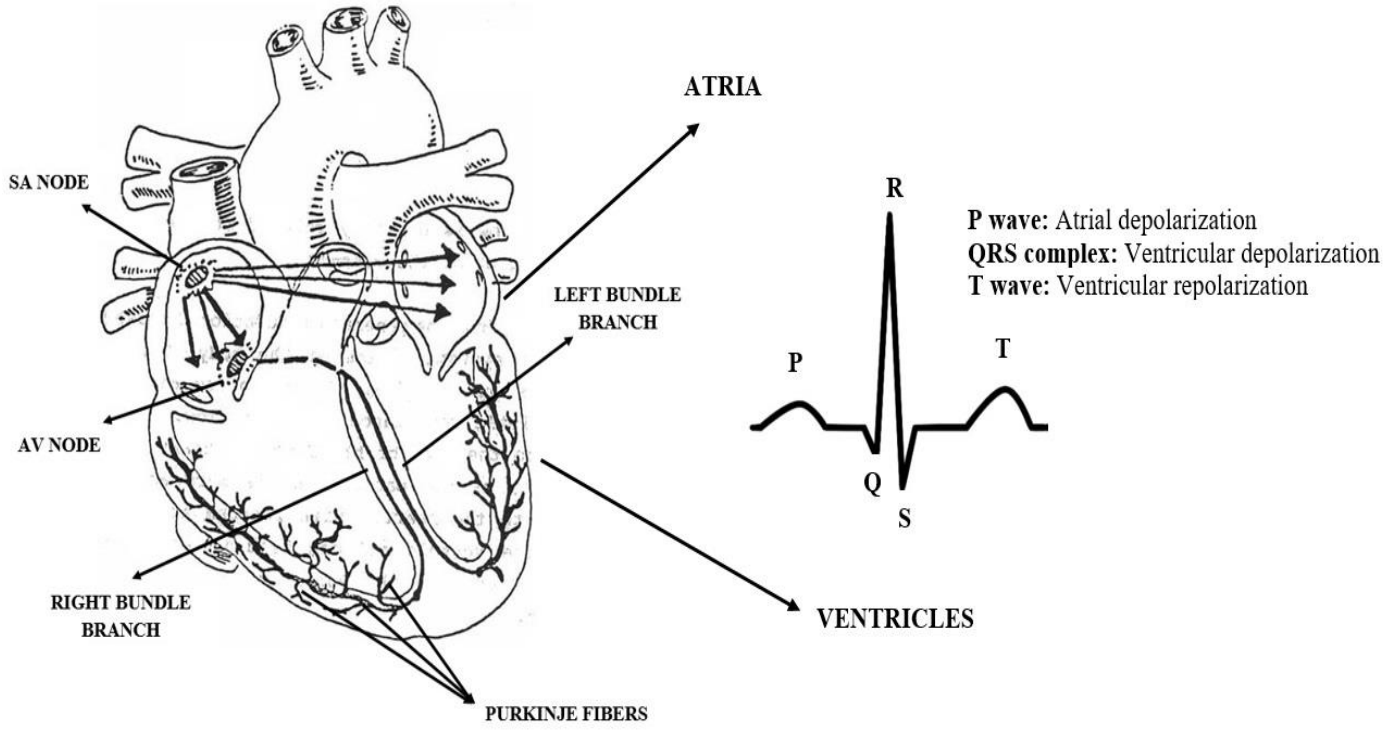


Figure 2. Electrical Conduction System of the Heart. Credit: nurseslearning.com

Ventricular systole starts at the beginning of the QRS complex and expands in the direction of the S-T segment (Kamath et al., 2012; Shaffer et al., 2014). Ventricular repolarization produces the T-wave approximately 0.4 sec after the P-wave occurs. Finally, ventricular diastole occurs 0.6 s after the start of the P-wave (Kamath et al., 2012; Shaffer et al., 2014). (Figure 2).

A dynamic interplay between the SNS and PNS systems is considered to be a sign of a healthy organism (Kamath et al., 2012; Shaffer et al., 2014). Variation in the HR is a demonstration of the net impact of the interaction between SNS and PNS (McCraty, 2015). The rate of SA node without any nervous and hormonal influence is around 100 to 120 bpm at rest (Kamath et al., 2012). However, the predominant branch of the ANS at rest, the PNS inhibits some of the electrical impulses of SA node and thereby resulting in the net resting HR of 60-100 bpm in healthy individuals (Kamath et al., 2012). This shows that the predominant neural output comes from the PNS while the SNS is tonically active (i.e., minimum baseline activity) at rest in health individuals (Kamath et al., 2012).

The PNS (originating from cranial vagal nerve) slows the HR by stimulating intrinsic cardiac conduction system that extends toward the SA node, AV, node, and atrial myocardium (Kamath et al., 2012; Shaffer et al., 2014). Elevated efferent stimulation in the nerves of the cardiac nervous system activates acetylcholine release, binding to muscarinic receptors (M2) located on the myocardial fibers (Kamath et al., 2012; Shaffer et al., 2014). This leads to a decrease in the depolarization of the SA and AV nodes, and thereby, slows the HR. The response to vagal innervation is instantaneous, takes place within a heart cycle, and therefore, impacts one or two heartbeat following its onset (Kamath et al., 2012; Shaffer et al., 2014). Once vagal innervation stops, the HR quickly decreases to its baseline level. The HR can also increase if the vagal innervation is diminished or blocked. Therefore, the difference in the time between sequential heartbeats are parasympathetically mediated (Kamath et al., 2012; Shaffer et al., 2014). Elevated efferent sympathetic nerves (originating from the T1-T4 segments of the spinal cord) stimulate the SA and AV nodes through the cardiac nervous system (Kamath et al., 2012; Shaffer et al., 2014). Action potentials resulting from sympathetic stimulation cause the release of norepinephrine,

binding to beta adrenergic receptors (beta 1) found on myocardial fibers (Kamath et al., 2012; Shaffer et al., 2014). This results in an increase in the depolarization of SA and AV nodes and thereby elevating HR (Kamath et al., 2012; Shaffer et al., 2014).

2.2. Overview of HRV

A healthy organism consists of a physiological control system that never rests nor operates in a static manner (Kamath et al., 2012; Shaffer et al., 2014). For instance; a healthy heart beats highly irregularly producing variation between one beat and the next as opposed to beating like a metronome with no variations created (Shaffer et al., 2014). Thus, HR is an indicative biomarker of the interaction between the sympathetic and parasympathetic divisions of the ANS (Kamath et al., 2012; Shaffer et al., 2014). The interaction between both branches is constantly occurring in an effort to reach a relatively stable environment interrupted by internal and external challenges (Kamath et al., 2012; Shaffer et al., 2014). This dynamic interaction between the PNS and SNS results in fluctuations in the length of the intervals between heartbeats or beat-to-beat changes, this is termed HRV. Afferent signals occurring from a various physiological processes including blood pressure oscillations, thermoregulation, respiration, and circadian rhythm also contribute to the variability in the HR (Kamath et al., 2012; Shaffer et al., 2014). Therefore, HRV can provide a window into cardiovascular and respiratory control mechanisms and as a means to examine the interaction between sympathetic and parasympathetic divisions of the ANS (Kamath et al., 2012; Shaffer et al., 2014).

2.2.1. Clinical Application of HRV

An optimum level of variability created by the regulatory systems of a body including the autonomic, cardiorespiratory, endocrine, and central nervous systems as well as chemoreceptors and baroreceptors is essential for a healthy organism (Heathers, 2014) Whereas a high level of

variability can be a sign of inefficient physiological functioning and energy utilization, low variability can be an indication of a pathology (Heathers, 2014) .

The clinical significance of the HRV was first reported by (Lee & Hon, 1965) when they examined fetal HRV during labor and delivery and its association with fetal health. They found that changes in HRV precedes fetal stress before any changes take place in the HR itself (Lee & Hon, 1965). Subsequently in 1970, it was shown that HRV predicted autonomic neuropathy in patients with diabetes prior to the beginning of symptoms (Ewing et al., 1976). Additionally, low HRV was linked with higher mortality in patients with acute myocardial infarction (Berntson et al., 2008), and the chance of sudden cardiac death in patients with acute myocardial infarction (Sessa et al., 2018).

Since then low HRV has been shown to be a valid and independent predictor of diverse pathological conditions including chronic renal failure (Axelrod et al., 1987), fibromyalgia (Kang et al., 2016), asthma (Kazuma et al., 1997), gastrointestinal disorders (Polster et al., 2018), congestive heart failure (Frenneaux, 2004), sudden cardiac death (Sessa et al., 2018), anxiety (Cohen & Benjamin, 2006), depression (Carney et al., 2001), and hypertension (Schroeder et al., 2003; Terathongkum & Pickler, 2004).

2.2.1.1. Effects of Hypertension on HRV

Hypertension is the most commonly observed cardiovascular disease risk factor in the United States (Arnett et al., 2019) as currently 46% of American adults have hypertension (Arnett et al., 2019), making it a significant health problem in the country (Arnett et al., 2019). Hypertension can increase the likelihood of developing coronary artery disease, stroke, renal failure, and cardiac heart failure (Clifford, 2002). However, the underlying etiology for the development of hypertension are largely unknown. One of the hypotheses regarding the initiation, progression, and

maintenance of hypertension is alterations in the neural control of the blood pressure (Kamath et al., 2012). These neural changes generally emerge as excessive sympathetic stimulation and parasympathetic inhibition (Esler, 2000). To a great extent, the current literature about HRV indicate that the vagal modulation of the HR is compromised in patients with hypertension (Clifford, 2002; Esler, 2000; Esler, 2009; Kamath et al., 2012).

HRV can provide important prognostic information in patients with hypertension. A number of studies investigated the prognostic importance of HRV in predicting the future risk of developing hypertension (Chakko et al., 1993; Esler, 2000; Kamath et al., 2012; Schroeder et al., 2003; Terathongkum & Pickler, 2004). The Atherosclerosis Risk in Communities study with a sample size of 12,000 showed that the HF ms^2 and SDNN were negatively correlated with incident hypertension after a 3-year follow-up period (Liao et al., 1996). In addition, those with the highest hypertension had lowest HF ms^2 and SDNN and highest LF/HF (Liao et al., 1996). Moreover, the second trial of the same project showed a negative correlation between the SDNN, RMSSD and the incident hypertension risk in the subjects followed for 9 years (Schroeder et al., 2003). Similar studies have further shown that patients with compromised ANS are at a high risk of developing hypertension. This is in line with the neural alterations (accentuated sympathetic and decreased parasympathetic activities) characterized with the early stages of hypertension (Julius, 1998; Kamath et al., 2012; Maver & Štrucl, 2004; Singh et al., 1998).

Antihypertensive medications, however, may be confounding factors in HRV analysis in patients with hypertension. It has been observed that patients with hypertension who use either β-blockers (Greenwood et al., 2000; Kamath et al., 2012), angiotensin-converting enzyme inhibitors (Binkley et al., 1993) or diuretics (Tsuji et al., 1996) had decreased HRV. However, the overall

impacts of single or particular combinations of antihypertensive medications on HRV in patients with hypertension are still debatable (Kamath et al., 2012).

2.3. HRV Signal Assessment

The HRV signal is usually measured by ECG recording detected over a certain period of time (McCraty, 2015; Peltola, 2012; Shaffer et al., 2014). HR monitors are also used for measuring HRV and many of those, including the most recent version Polar V800^{TM}, have been validated (Caminal et al., 2018; Giles et al., 2017; Giles et al., 2016). The algorithms of Polar HR monitors scan the raw data and identifies particular patterns of data such as the R-wave in the ECG signal (Nabil & Reguig, 2015). As the first R-peak is captured, the algorithm generates the time between this and the subsequent R-waves, which is called R-R intervals that are used to calculate HRV measures (Nabil & Reguig, 2015). This process of calculating R-R intervals repeats until the recording finishes.

The R-wave of QRS complex is utilized mostly as the fiducial point because of its distinct amplitude (De Geus et al., 2019; McCraty, 2015; Nabil & Reguig, 2015; Peltola et al., 2008; Shaffer et al., 2014). However, the true marker of the sinus rhythm is considered the P-wave, as the P-wave is the closest indicator of atrial depolarization than the R-wave on ECG (De Geus et al., 2019; McCraty, 2015; Nabil & Reguig, 2015; Peltola et al., 2008; Shaffer et al., 2014). In practice, the P-wave is too small to accurately detect it by the computer algorithms, particularly in the presence of artifacts though in theory, it would be more appropriate to utilize P-P intervals for HRV analysis (De Geus et al., 2019; McCraty, 2015; Nabil & Reguig, 2015; Peltola et al., 2008; Shaffer et al., 2014). However, several lines of evidence have shown that changes in R-R intervals reflect the SA node rhythms with high accuracy (De Geus et al., 2019; McCraty, 2015; Nabil & Reguig, 2015; Peltola et al., 2008; Shaffer et al., 2014). Therefore, the accurate R-wave detection

is of crucial importance in the obtaining of the reliable HRV outcomes. In other words, the accuracy of the R-wave detection is correlated with the lower number of artifacts in R-R intervals, and thus, improved HRV analysis.

The general order of obtaining the time series of the R-R interval formation is as follows: 1) the difference between each sequential R-R intervals is calculated; and 2) length of the sequential R-R intervals are defined, forming the discrete time series of R-R intervals (Peltola, 2012). The time series of the R-R intervals are not sampled at fixed intervals because of the varying differences in the length of the neighboring R-R intervals (Peltola, 2012). Not equidistantly sampled R-R intervals are problematic for frequency domain measures as the power spectral analysis requires equally spaced R-R intervals (Peltola, 2012). Several methods have been recommended to avoid this issue prior to the power spectral analysis. A detailed description of each methods can be found elsewhere (Nabil & Reguig, 2015; Peltola, 2012) as it is beyond the scope of this thesis.

Another important factor for obtaining accurate HRV measures is the sampling rate of the data acquisition system (e.g., ECG, HR monitor). Higher sampling frequency ensures the better resolution of the R-R intervals (Peltola, 2012; Shaffer et al., 2014). Lower sampling frequency may generate inaccurate HRV measures if the overall variability is abnormally low such as in patients with heart failure (Sessa et al., 2018; Shaffer et al., 2014). The recommended sampling frequency is at least 500-1000 Hz (Peltola, 2012; Shaffer et al., 2014).

In 1996, the task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology reported three methods for HRV signal assessment: 1) time domain analysis; 2) frequency domain analysis or power spectral density analysis; and 3) non-linear analysis (Malik et al., 1996).

2.3.1. Time Domain Measures

Time domain measures quantify the amount of HRV during the entire recording and are divided into statistical and geometric measures. Table 1 shows the most commonly reported statistical and geometric time domain measures as well as brief descriptions.

Table 1. Statistical and geometric time domain measures

Variables	**Units**	**Description**
SDNN	ms	Standard deviation of normal R-R intervals
RMSSD	ms	Root mean square of successive differences normal R-R intervals
pNN50	%	Percentage of successive normal R-R intervals greater than 50 ms
HRV triangular index	-	Integral of the density of the R-R interval histogram divided by its height
TINN	ms	Triangular interpolation of R-R intervals

2.3.1.1. Statistical Measures

Calculations of statistical time domain measures are based on beat-to-beat changes, and thus, are considered sensitive to outliers and artifacts. Statistical time domain measures (Table 1) from different studies can be compared with one another provided that the recording time is identical, and the data are obtained under the similar laboratory conditions (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014).

The *SDNN* reflects all regular or periodic fluctuations that contribute to the HRV, including the slow oscillations that reflect the intrinsic ability of the heart react to hormonal stimulants (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). The SDNN is correlated with VLF, LF, and total power (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). The actual values of SDNN change depending on the recording length; when the recording is longer, then the SDNN

values become higher and vice versa (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). Therefore, SDNN measures acquired from different recording lengths should not be compared with each other (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). Further, it is considered that SDNN is more accurate when analyzed over longer recordings (24 hr) than shorter recordings (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014).

The *RMSSD* measures the high frequency variations of the HR and reflects the parasympathetic influence on the heart (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). The RMSSD is strongly correlated with pNN50%, HF ms^2, and SD1 (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). While the RMSSD is less influenced by respiration than is respiratory sinus arrhythmia, the RMSSD is more influenced by the parasympathetic activity than the SDNN in general (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014).

The *pNN50%* is strongly correlated with RMSDD and HF ms^2, reflecting the influence of parasympathetic activity (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). However, most researchers prefer to report RMSSD over pNN50% in the time domain measures because it is thought that RMSSD provides better assessment of the respiratory sinus arrhythmia (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014).

2.3.1.2. Geometric Measures

The basis of the geometric measures is a histogram of the frequency distribution. When the frequency distribution appears tight and high, it means that all R-R intervals remain within a small range of values, and thus HRV is low (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). However, when the frequency distribution appears wide and low, it means that R-R intervals remain within a large range of values, and thus HRV is high. Geometric time domain measures (Table 1) quantify the shape of the histogram of the R-R tachogram, presenting R-R intervals in

geometric patterns (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). These measures are considered insensitive to outliers, and thus, are more likely to be free of artifacts and software errors in obtaining accurate R-R intervals (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). In addition, they are recommended to be used only for long-term (usually 24hr) recordings as the evidence indicates that they may underestimate overall HRV of short-term recordings (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). Table 4 shows the most commonly reported geometric time domain measures as well as a brief description.

The *HRV index* is a measure that captures the duration of the R-R intervals that acts as the x-axis and the number of each R-R interval that acts as the y-axis of the plot (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). The HRV index is calculated by taking the integral of the density of the R-R interval histogram (i.e., total number of R-R intervals) and dividing it by its height. Since the results depend on the width of the frequency histogram bin, the width of this bin is recommended to be set to 1/128 or 7.8 ms (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014).

The *TINN* is the baseline width of the normal R-R interval histogram in ms that is assessed through triangular interpolation. The TINN and HRV index are correlated with SDNN since all three variables reflect the total variance of HRV (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014).

2.3.2. Frequency Domain Analysis

The power spectral analysis was established in 1981 by Akselrod et al. for the purpose of decomposing the HRV waveform into its component rhythms, each representing a specific physiological influence (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). This finding of breaking down HRV by power spectral analysis dominated HRV research throughout the 1980s

(Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). Power spectral analysis can be measured by fast Fourier transform model (non-parametric) or autoregressive model (parametric) (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). While the non-parametric fast Fourier transform model is fast and straightforward, it may cause spectra with many spikes for short HRV recordings (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). Parametric autoregressive model, on the other hand, is smoother and more appropriate for short HRV recordings although it is complicated to establish the optimal autoregressive model order (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). Regardless of the methods, the calculation of power spectral analysis assumes an equally spaced time base, unlike an N-N interval base with variation between beats (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014).

Unlike time domain measures, power spectral analysis allows to split the waveform, thereby revealing the contribution of the separate physiological regulatory mechanisms on HRV that act at specific frequencies (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). Frequency is the number of events occurring repeatedly per unit of time. Its unit of measures is the Hz where 0.1 Hz indicates that an event repeats once per every 10 seconds (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). Table 2 contains the most commonly reported statistical and geometric time domain measures as well as brief descriptions.

Table 2. Frequency time domain measures

Variables	**Units**	**Range (Hz)**	**Description**
VLF	ms^2	< 0.04	Reflects the circadian rhythms, thermoregulation, and peripheral vasomotor influences
LF	ms^2	0.04-0.15	Reflects sympathetic and parasympathetic nervous systems as well as baroreceptors
HF	ms^2	0.15-0.40	Reflects parasympathetic influence and fluctuations caused by respiration
LF/HF	-	-	Reflects the sympathovagal balance

VLF band requires at least 5 min of recording, but 24 hr recording is considered to be optimal. The reason for the recording requirement for VLF ms^2 is that it represents overall activity of the various slow physiological fluctuations reported in Table 2 and thus, it cannot be accurately estimated with 5 min or less of recording time (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014).

LF band represents the physiological influences reported in Table 2. The PNS can produce oscillations down to 0.05 Hz, whereas the SNS does not oscillate until >0.1 Hz. Slow respiration can produce HR oscillations that cross over into the LF ms^2. Hence, respiration driven vagal influences can be part of the LF ms^2 if respiration frequency is <8 breaths per minute or if a person sighs or takes a deep breath (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014).

HF band represents the physiological influences reported in Table 2. Also, the HF band is called the respiratory band since it correlates with HR rhythms influenced by the respiratory sinus arrythmia (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014).

LF/HF ratio represents the physiological influences reported in Table 2. Although it still a matter of debate a low and high LF/HF ratio is considered to reflect sympathetic and parasympathetic dominance, respectively. Changes in LF/HF ratio may be either because of an increased sympathetic tone or parasympathetic withdrawal (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014).

2.3.3. Non-linear Analysis

The requirement for the time and frequency domain measures is that R-R intervals should be stationary. Non-stationary data indicate sudden variations in R-R intervals, mostly seen as response to exercise, posture change, or pathology such as hypertension (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014).

Abrupt changes frequently occur in the ANS activity due to a variety of physiological or environmental stimuli, indicating the complexity of the heart rhythms (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). Non-linear measures are used to calculate the complexity of heart rhythm by using the Poincare plot, which is a scatter plot where each R-R interval is plotted against the next R-R interval (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). In this way, a graphical representation of cardiovascular rhythms is provided, appearing in an elliptical shape (Heathers, 2014; Maud & Foster, 2006; Shaffer et al., 2014). Table 3 shows the most commonly reported non-linear HRV measures and a brief description.

Table 3. Non-linear measures

Variables	**Units**	**Description**
SD1	ms	Reflects short-term variability and is correlated with RMSSD and HF ms^2
SD2	ms	Reflects long-term variability and is correlated with LF ms^2
SD2/SD1	-	Reflects the unpredictability of R-R interval time series and is correlated with LF/HF ratio.

2.4. Artifacts in R-R Intervals

Ideally, R-R intervals should consist of sinus rhythms (i.e., normal R-R intervals) to acquire accurate and reliable time, frequency, and non-linear HRV measures. However, the R-R intervals obtained from HR monitors are rarely perfect. Indeed, Polar HR monitors including the S810™ series, RS800G3™, and the RS800CX™, and the recent version of Polar V800™ showed varying percent of artifact/error rates: Polar S810i™ (6.93%) in healthy adults (Vanderlei et al., 2008), S810™ (0.40%) in active healthy adults (Gamelin et al., 2006), S810™ (0.32%) (Kingsley et al., 2005) in healthy adults, and the recent version of Polar V800 (0.08%) (Giles et al., 2016) and (0.10%) (Giles et al., 2017) in supine position; and the Polar V800™ (0.08% and 0.06%) in healthy

adults in standing position (Giles et al., 2016; Giles et al., 2017). The presence of artifacts can considerably distort the HRV signal, and thus, result in erroneous generation of the HRV signal. Distorted R-R intervals can easily be distinguished as their length is many times larger or smaller than the sinus rhythm intervals (Nabil & Reguig, 2015). Artifacts in R-R intervals are classified by technical and physiological origins.

Artifacts with technical origin can result from a variety of reasons. For example; muscle contractions nearby the heart, especially from chest and arm muscles can distort the R-R intervals because of the electrical activity created during patient motion (Nabil & Reguig, 2015). Additionally, loose contact between the skin and chest strap or electrodes or sweating during the recording can cause artifacts with technical origin (Nabil & Reguig, 2015). Moreover, detection algorithms may fail to correctly identify the R-waves, causing sudden variations in the HRV (Nabil & Reguig, 2015). Presence of wireless networks that can interfere the Bluetooth transmission of HR monitors during the recording can also produce artifacts with technical origin (Giles et al., 2017).

Artifacts with physiological origins appear when the usual electrical activity of the heart is impaired due to various reasons. Normal heart rhythms are the result of regular electrical impulses originating from SA node (Peltola et al., 2012). When the origin of electrical impulses derives from the outside of the SA node, irregular and abnormal beats are produced such as ectopic (premature) and atrial or ventricular fibrillation (Peltola et al., 2012). These beats originate from atria or ventricles rather than SA node. When the origin of abnormal beats is from atria it is termed pre-mature atrial contraction, while it is termed pre-mature ventricular contraction when the origin of the beat is the ventricles (Peltola et al., 2012). Ectopic beats introduce signal ambiguity as they cause preceding and successive R-R intervals to differ from each other (Nabil & Reguig, 2015).

The appearance of ectopic beats in the time series of R-R intervals is usually viewed as a short R-R interval followed by a long R-R interval compared to sinus rhythm intervals or vice versa (Figure 5 and 6) (Nabil & Reguig, 2015). Therefore, the presence of ectopic beats can introduce extensive source of error into HRV measures (Nabil & Reguig, 2015). Although vast majority of persons experience ectopic beats, patients with cardiovascular diseases (e.g., myocardial infarction) tend to have more frequent (90-95%) artifacts with physiological origin as this population is more vulnerable to premature ventricular and atrial contractions (Kamath and Fallen, 1995; Peltola et al., 2012). However, technical artifacts are commonly observed with HR monitors regardless of the type of population.

Artifacts with physiological origins and technical origins can appear simultaneously in the time series of R-R intervals. Therefore, it is difficult to distinguish artifacts with physiological origins from artifacts with technical origins (Giles et al., 2017); ectopic beats can look identical to the following artifact types, particularly as a T1, T2, T3, or T4, which are defined under *2.4.1 Types of Artifacts in R-R intervals* (Nabil & Reguig,2015; Giles et al., 2017).

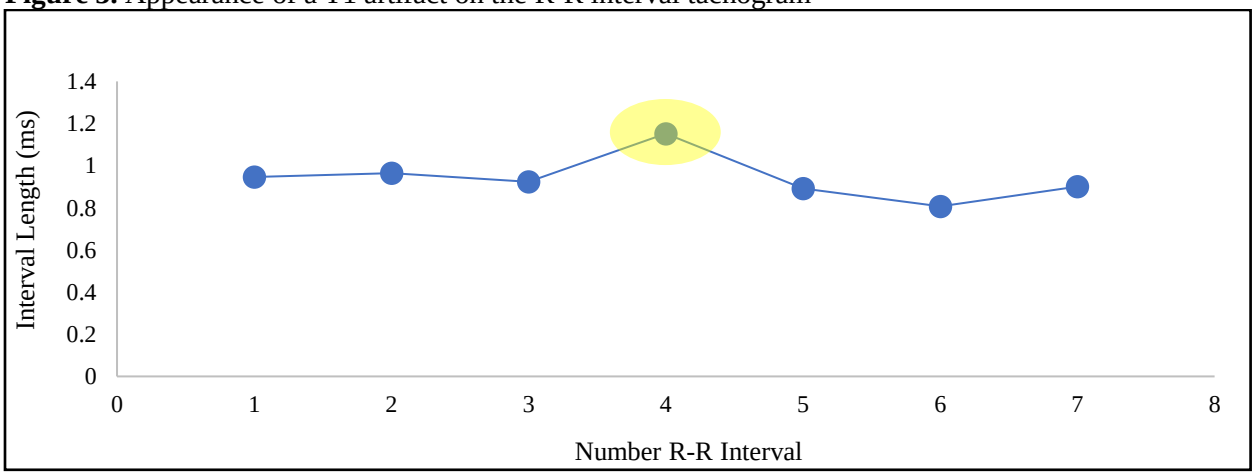
2.4.1. Types of Artifacts in R-R Intervals

Initially, five types of artifact descriptions were developed by (Gamelin et al., 2006) that included T1 through T5. Later, two more artifact descriptions were added by (Giles et al., 2016) after using Polar V800^{TM} HR monitor in healthy individuals that include T6a and T6b. The descriptions of all seven artifacts from T1 to T6b are present below (Giles et al., 2016):

Single Interval of Discrepancy (T1): T1 is defined as a positive or negative single interval difference > 20 ms (Figure 3). Artifact T1 is undetectable without simultaneous ECG recording because these may be the result of changes in interval length (Figure 3). An example of the appearance of T1 in an R-R interval time series would be as follows:

Time	R-R Intervals
0. 946	0. 946
1.911	0. 965
2.834	0. 923
3.986	1. 152 [T1]
4.878	0. 892
5.685	0. 807
6.586	0. 901
.	.
.	.
.	.

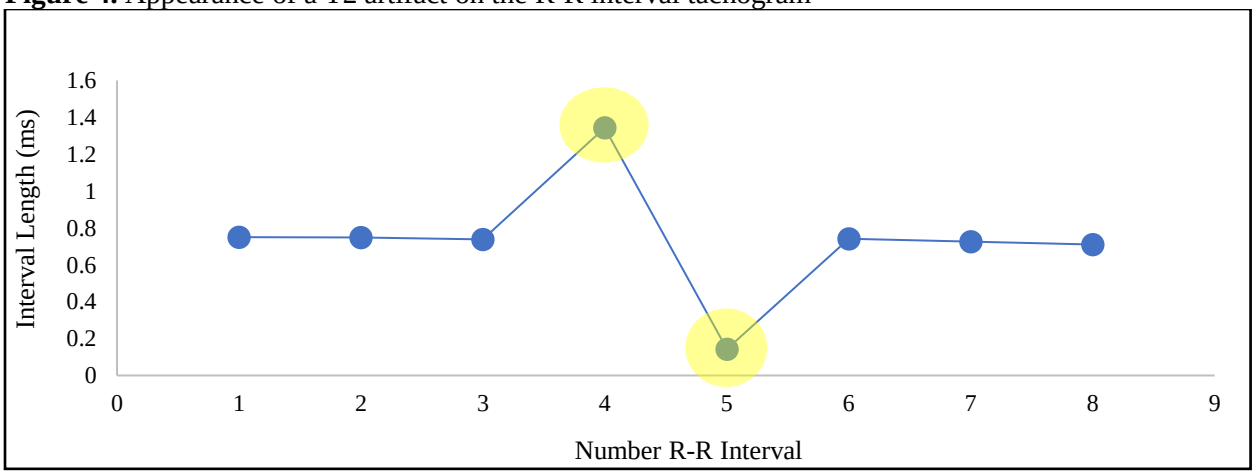
Figure 3. Appearance of a T1 artifact on the R-R interval tachogram



Long interval and short interval (T2): T2 is defined as a long interval followed by a short interval while the two R-R intervals on either side were < 20 ms (Figure 4). An example of the appearance of T2 in R-R interval time series would be as follows:

Time	R-R Intervals
247.513	0. 750
248.261	0. 748
248.999	0. 738
250.342	1. 343 [T2]
250.485	0. 143
251.226	0. 741
251.952	0. 726
252.663	0.711
.	.
.	.
.	.

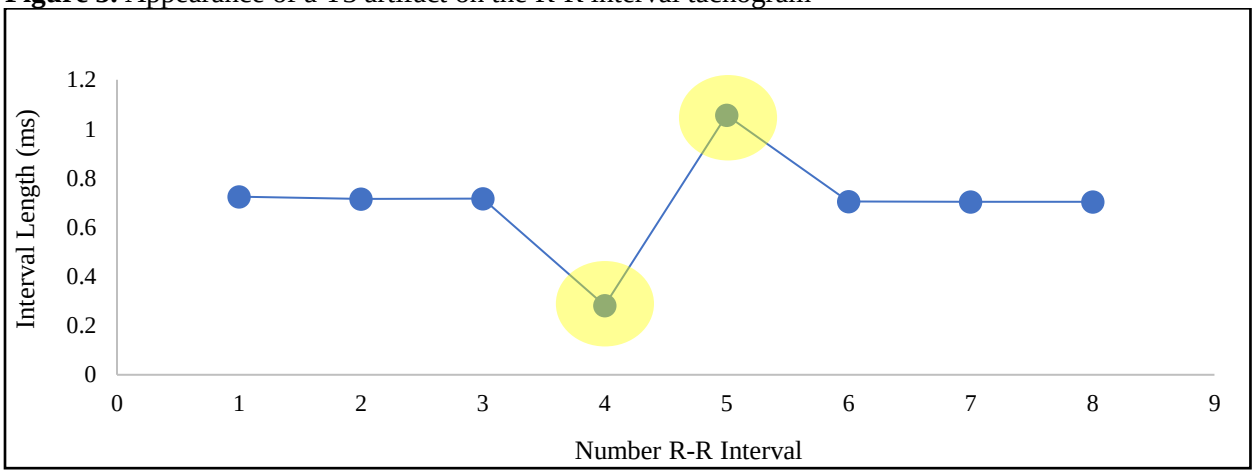
Figure 4. Appearance of a T2 artifact on the R-R interval tachogram



Short interval and long interval (T3): T3 is defined as a short interval followed by a long interval while the two R-R intervals on either side < 20 ms (Figure 5). An example of the appearance of T3 in R-R interval time series would be as follows:

Time	R-R Intervals
131.622	0. 725
132.337	0. 715
133.053	0. 716
133.335	0. 282 [T3]
134.391	1. 056
135.116	0. 725
135.830	0. 714
136.573	0. 743
.	.
.	.
.	.

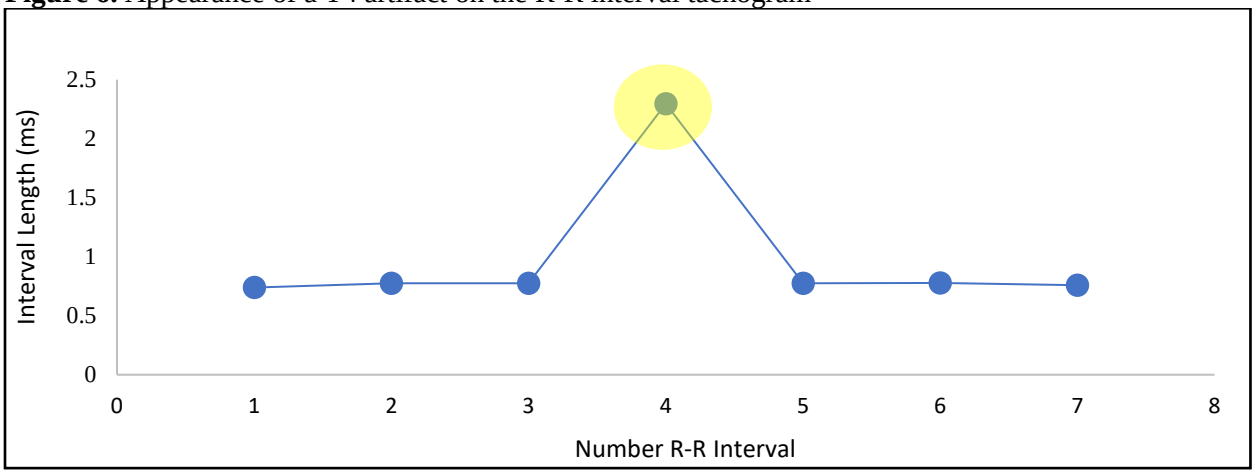
Figure 5. Appearance of a T3 artifact on the R-R interval tachogram



Too few intervals detected (T4): This is considered T4 and defined as missed beats equivalent to two or three ECG R-R intervals (Figure 6). An example of the appearance of T4 in R-R interval time series would be as follows:

Time	R-R Intervals
24.245	0. 739
25.021	0. 776
25.795	0. 774
28.091	2.296 [T4]
28.865	0. 774
29.643	0. 778
30.403	0. 760
.	.
.	.
.	.

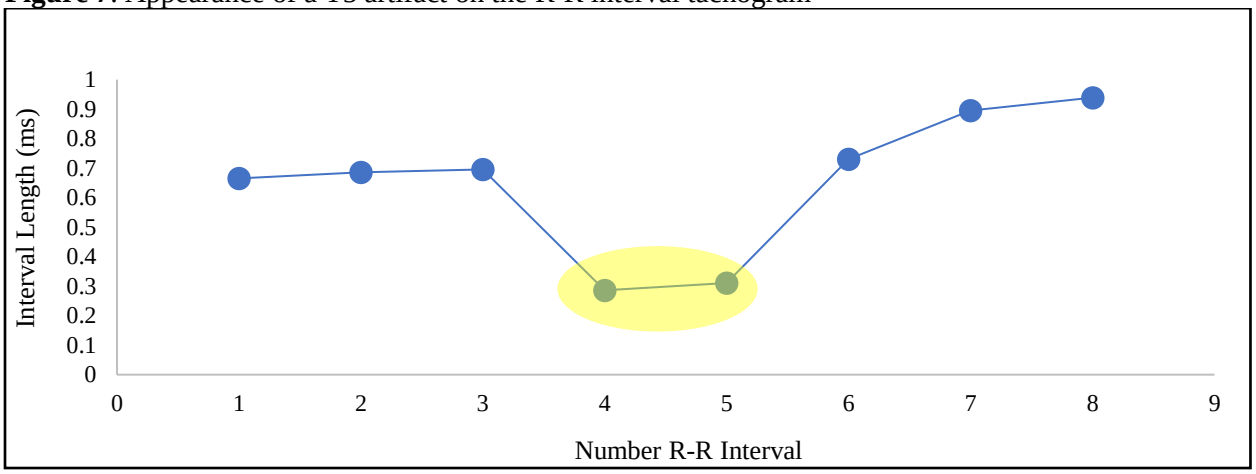
Figure 6. Appearance of a T4 artifact on the R-R interval tachogram



Too many intervals detected (T5): T5 is defined as extra short beat equivalent to one ECG R-R interval (Figure 7). An example of the appearance of T5 in R-R interval time series would be as follows:

Time	R-R Intervals
157.670	0. 665
158.356	0. 686
159.052	0. 696
159.338	0. 286 [T5]
159.649	0. 311
160.379	0. 730
161.274	0. 895
162.213	0. 939
.	.

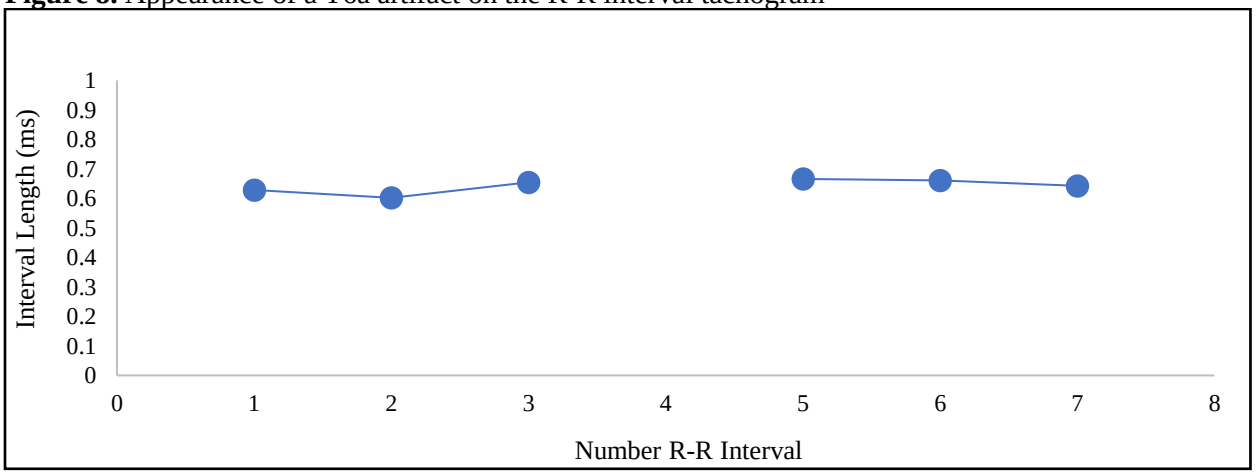
Figure 7. Appearance of a T5 artifact on the R-R interval tachogram



Entirely missed beats (undetectable) (T6a): T6a is defined as entirely missed beats while there is no difference in the HR monitor time stamp. Artifact T6a is undetectable without simultaneously measured ECG recording (Figure 8). An example of the appearance of T6a in R-R interval time series would be as follows:

Time	Difference	R-R Intervals	
134.041	0. 629	0. 629	
134.643	0. 602	0. 602	
135.298	0. 655	0. 655	
.	.	.	[T6a]
135.964	0. 666	0. 666	
136.625	0. 661	0. 661	
137.268	0. 643	0. 643	
.	.	.	
.	.	.	
.	.	.	

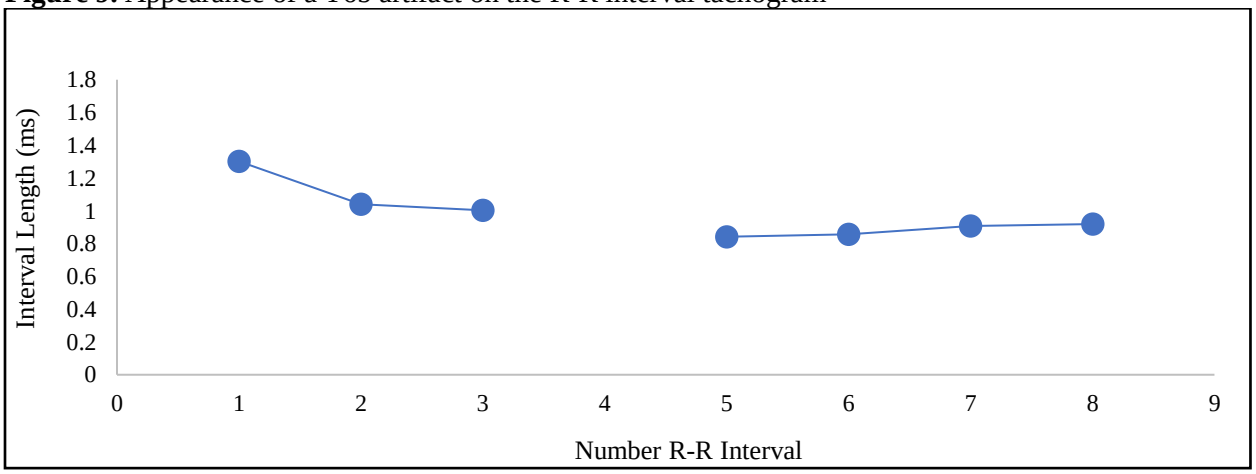
Figure 8. Appearance of a T6a artifact on the R-R interval tachogram



Entirely missed beats (detectable) (T6b): T6b is defined as entirely missed beat while there is no difference in the HR monitor time stamp. Artifact T6b is detectable without a simultaneously measured ECG recording because a larger than expected gap in the time stamp is present (Figure 9). An example of the appearance of T6b in R-R interval time series would be as follows:

Time	Difference	R-R Intervals
55.840	1. 302	1. 302
56.881	1. 041	1. 041
57.884	1. 003	1. 003
.	.	. [T6b]
59.841	**1. 957**	**0. 842**
60.698	0. 857	0. 857
61.605	0. 907	0. 907
.	.	.
.	.	.
.	.	.

Figure 9. Appearance of a T6b artifact on the R-R interval tachogram



The current literature shows that the most common artifact type that was detected by the Polar S810i™, S810™, RS800CX™, and V800™ was T4 (missed beats) with the ratio varying around 60-90% of the total number of errors in the supine and standing positions (De Rezende et al., 2016; Gamelin et al., 2006; Giles et al., 2017; Giles et al., 2016; Kingsley et al., 2005; Vanderlei et al., 2008; Vasconcellos et al., 2015). Even a single artifact can dramatically change HRV measures, particularly frequency domain variables; a T4 artifact or and ectopic beat has been

shown to increase HF ms^2 and LF ms^2 approximately 3-fold in HRV recordings in supine position (Berntson & Stowell, 1998; Nabil & Reguig, 2015). In addition, technical and physiological artifacts have been shown to increase SDNN, RMSSD, and pNN50% by approximately 2-fold (Bruggemann et al., 1993; Nabil & Reguig, 2015). Therefore, artifacts create a serious problem for obtaining accurate HRV measures and significantly increase the chance of ANS misinterpretation in healthy and unhealthy populations. The presence of a higher number of artifacts in clinical populations than that of healthy individuals makes the adults with hypertension more vulnerable to ANS misinterpretation. Therefore, correction of artifacts with an appropriate method is essential to prevent potential misinterpretation of ANS that may result in incorrect diagnosis of adults with hypertension.

2.4.2. Precautions for R-R Intervals Free of Artifacts

The number of artifacts in R-R intervals can be minimized with a well-controlled laboratory environment and following standard procedures before, during, and following the HRV recording. In a well-controlled laboratory environment, the following precautions should be taken (Malik et al., 1996): 1) HRV measurement should be carried out at the same time of a day because of the circadian rhythm that naturally causes changes in autonomic balance such as the morning and evening; 2) avoiding too bright a light or noise; 3) maintaining optimum room temperature; and 4) preparing the skin (i.e., shaving hair on the chest if necessary) for electrode attachment. Pre-measurement precautions include (Malik et al., 1996): 1) avoiding caffeine or smoking at least 48 hr before the experiment; 2) avoiding the HRV measurement immediately after a meal (at least 2 hr required); and 3) at least 5 min pre-measurement resting period for the subjects to adjust themselves to the new environment. During experiment measurement precautions include (Malik et al., 1996): 1) maintaining a comfortable measurement position (e.g., supine, sitting, or standing);

2) avoiding movement, talking, or sleeping; and 3) avoiding intentional controlling of breathing. Collecting the R-R intervals free of artifacts, however, can still be quite challenging in the case of uncooperative subjects or long-term HRV measurements (Malik et al., 1996). Regardless of the all precautions, recording of absolutely clean and artifact-free data is difficult. Thus, it is necessary to review the recording and carefully correct any artifacts before calculating HRV measures to ensure accurate and reliable data.

2.5. Correction of R-R intervals with Artifacts

As mentioned previously, artifacts can significantly distort time, frequency, and non-linear HRV measures, especially frequency domain measures. When artifacts are present, the first option should be to select artifact-free segments of the R-R interval time series (Peltola, 2012). Clean segments of the R-R interval time series, however, should include no less than 2.5 min to ensure the accurate calculation of power distribution within a frequency domain (Peltola, 2012). If the ratio of distorted R-R intervals is <10% (or N-N intervals ≥ 90%) the artifacts can be replaced by different correction methods before the calculation of HRV measures (Peltola, 2012). However, if the ratio of distorted R-R intervals is > 20%, rejection of entire recording may be necessary (Peltola, 2012).

2.5.1. Manual Correction

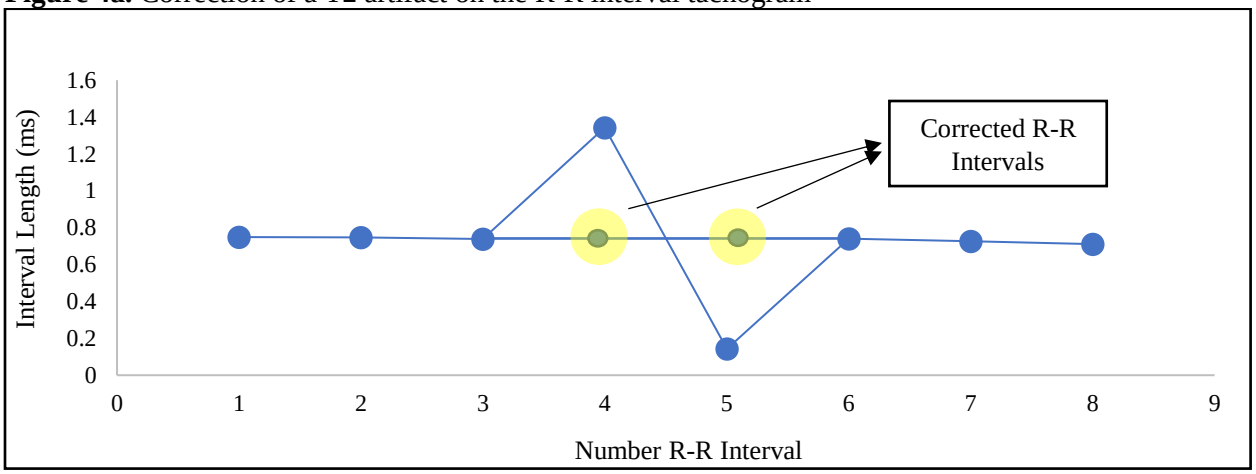
Artifact correction can be performed manually or automatically using various HRV software packages. The general consensus regarding the more accurate method for correcting artifacts is the MC with visual confirmation of the R-R intervals and the appropriate correction method. The MC cannot be truly replaced with automatic correction software packages (Peltola, 2012). First, (Gamelin et al., 2006) developed the guideline of methods of correction for T1 through T5. Later, (Giles et al., 2016) updated it by adding T6a and T6b after their validation of the Polar V800™

HR monitor among healthy individuals. Previously, Gamelin et al., (2006 and Gamelin et al., (2008) corrected all artifacts from T1 to T5. However, (Giles et al., 2016) argued that correction of T1, a difference of 20 ms at a single interval (<50% increase in length over the adjacent intervals) could be either the result of an actual artifact or random fluctuation in interval length. Therefore, the detection of a T1 artifact would be impossible without the simultaneously recorded ECG. Similarly, Giles et al., (2016) concluded that T6a cannot be visible without simultaneous ECG recording as there seems to be no change in the HR monitor time stamp or R-R intervals when visually checked. Therefore, only identifiable artifacts (T2-T5, T6b) are recommended to be corrected following the guidelines below (Giles et al., 2016):

Long interval and short interval: T2 is corrected by averaging the long and short intervals and the result is replaced with both intervals (Figure 4a). An example of a T2 correction artifact is as follows: 1.343 (long interval) + 0.143 (short interval) / 2 = 0.743.

Time	**R-R Intervals**	**Correction**
247.513	0. 750	0. 750
248.261	0. 748	0. 748
248.999	0. 738	0. 738
250.342	1. 343 [T2]	0. 743
250.485	0. 143	0. 743
251.226	0. 741	0. 741
251.952	0. 726	0. 726
252.663	0. 711	0. 711
.	.	.
.	.	.

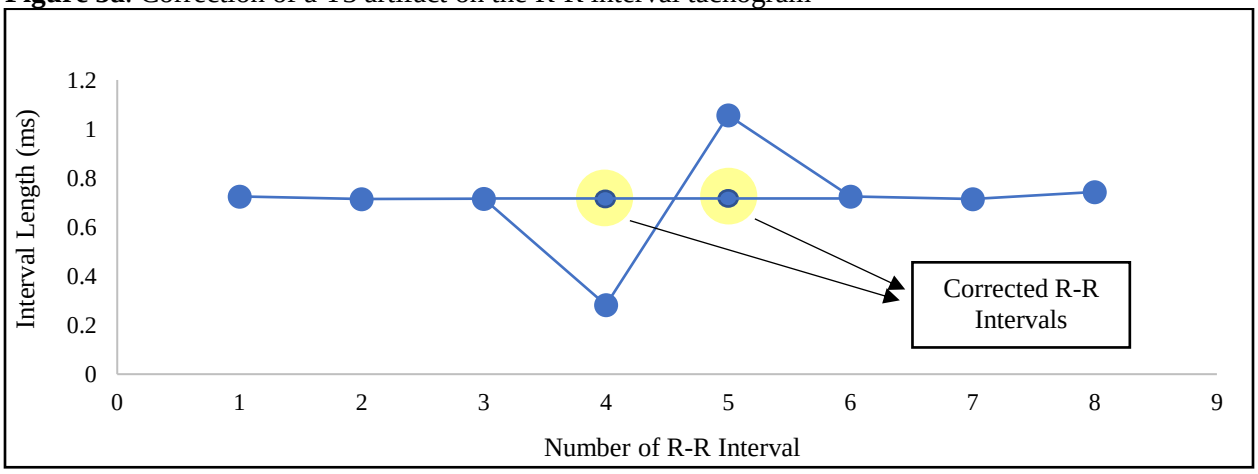
Figure 4a. Correction of a T2 artifact on the R-R interval tachogram



Short interval and long interval: T3 is corrected by averaging the short and long intervals and the result is replaced with both intervals (Figure 5a). An example of a T3 correction is as follows: 0.282 (short interval) + 1.056 (long interval) / 2 = 0.669.

Time	R-R Intervals	Correction
131.622	0. 725	0. 725
132.337	0. 715	0. 715
133.053	0. 716	0. 716
133.335	0. 282 [T3]	0. 669
134.391	1. 056	0. 669
135.116	0. 725	0. 725
135.830	0. 714	0. 714
136.573	0. 743	0. 743
.	.	.
.	.	.
.	.	.

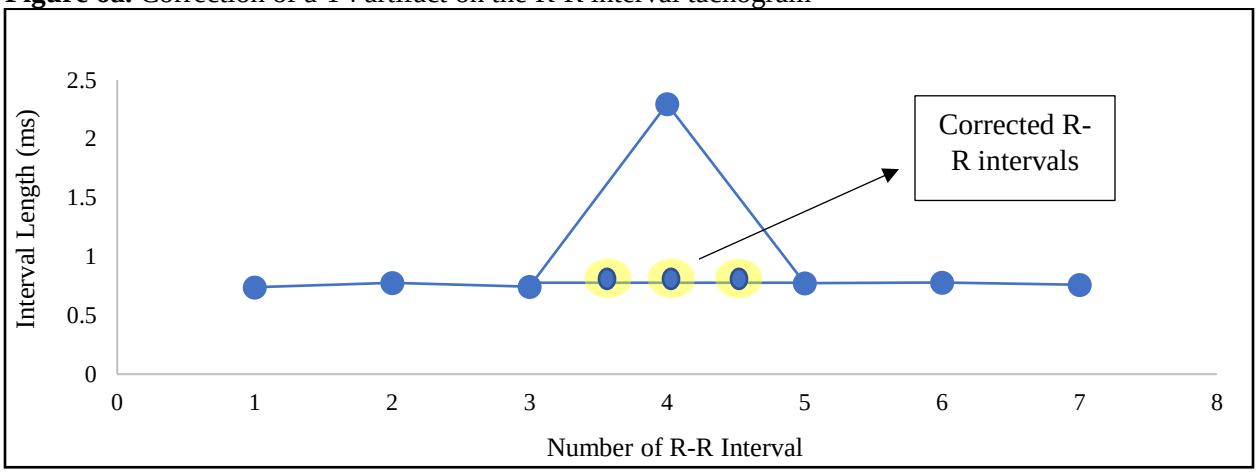
Figure 5a. Correction of a T3 artifact on the R-R interval tachogram



Too few intervals detected: T4 is corrected by dividing artificially long R-R interval by the number of missed beats and the result is replaced with the long interval however many missed beats are present (Figure 6a). An example of a T5 correction is as follows: 2.296 (artificially long R-R interval) / 3 (missed beats) = 0.765

Time	**R-R Intervals**	**Correction**
24.245	0. 739	0.739
25.021	0. 776	0.776
25.795	0. 774	0.744
28.091	2.296 [T4]	0.765
28.865	0. 774	0.765
29.643	0. 778	0.765
30.403	0. 760	0.774
.	.	0.778
.	.	0.760
.	.	.

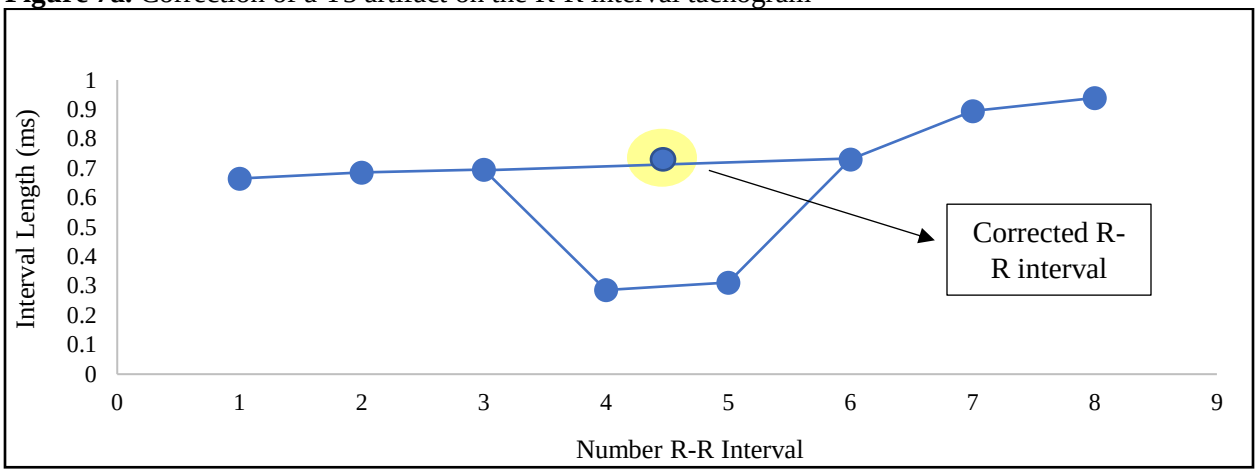
Figure 6a. Correction of a T4 artifact on the R-R interval tachogram



Too many intervals detected: T5 is corrected by adding two extra short R-R intervals together and the result is replaced with both intervals (Figure 7a). An example of a T5 correction is as follows: 0.286 (extra short R-R interval) + 0.311 (extra short interval) = 0.597

Time	R-R Intervals	Correction
157.670	0. 665	0. 665
158.356	0. 686	0. 686
159.052	0. 696	0. 696
159.338	0. 286 [T5]	0. 597
159.649	0. 311	0. 730
160.379	0. 730	0. 895
161.274	0. 895	0. 939
162.213	0. 939	.
.	.	.
.	.	.
.	.	.

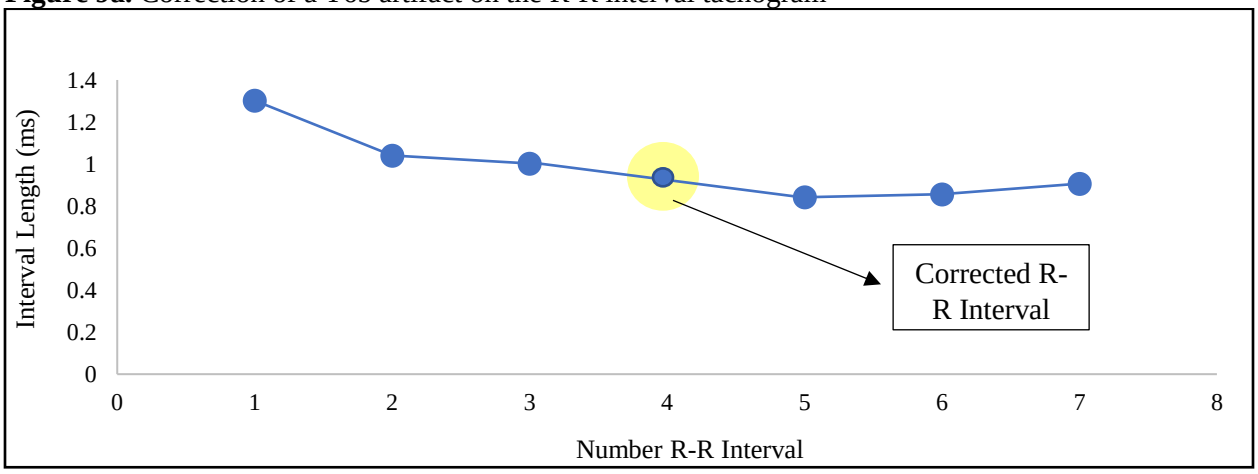
Figure 7a. Correction of a T5 artifact on the R-R interval tachogram



Entirely missed beats (detectable): T6b is corrected by subtracting the known interval from the difference in the time stamp, and the result is replaced with the entirely missed R-R interval and the known interval (Figure 9a). An example of a T6b correction is as follows: 1.957 (difference in the time stamp) – 0.842 (the known interval) = 1.115

Time	Difference	R-R Intervals	Correction
55.840	1. 302	1. 302	1. 302
56.881	1. 041	1. 041	1. 041
57.884	1. 003	1. 003	1. 003
.	.	. [T6b]	1. 115
59.841	**1. 957**	**0. 842**	1. 115
60.698	0. 857	0. 857	0. 857
61.605	0. 907	0. 907	0. 907
.	.	.	.
.	.	.	.
.	.	.	.

Figure 9a. Correction of a T6b artifact on the R-R interval tachogram



2.5.2. Additional Manual Correction Methods

Other manual correction methods are available in the current literature including deletion, degree zero, degree one, and cubic spline interpolations (Nabil & Reguig, 2015; Peltola, 2012) Interpolation methods (degree zero, degree one, cubic, cubic spline) can be performed using programming languages comprising MATLAB, Phyton, SciLab, etc. A brief description of these methods and their impact on HRV measures are discussed below.

In the deletion method, the distorted R-R intervals are eliminated, and previous normal R-R intervals are moved to take place of the deleted R-R intervals (Nabil & Reguig, 2015; Peltola, 2012). However, deletion of R-R intervals shortens the length of R-R interval time series, which can lead to an unacceptable bias in HRV measures, particularly with LF ms^2 and HF ms^2 (Nabil & Reguig, 2015; Peltola, 2012). Unlike the deletion, distorted R-R intervals are replaced with normal R-R intervals in the interpolation methods (Nabil & Reguig, 2015; Peltola, 2012). In these methods, therefore, the length of the R-R interval time series remains the same, reducing the chance of bias in HRV measures (Nabil & Reguig, 2015; Peltola, 2012). In the *interpolation of degree zero method*, artifacts are replaced with an average R-R interval that is calculated from adjacent R-R intervals (Nabil & Reguig, 2015; Peltola, 2012). When used on large sections of

artifacts, the degree zero causes a flat shape on R-R interval tachogram, an inaccurate trend, and significant biases in LF ms^2 and VLF ms^2 as the method calculates the same averaged R-R interval over a whole segment (Nabil & Reguig, 2015; Peltola, 2012). In the *linear interpolation* method, a straight line is placed over the artifacts to acquire new R-R intervals (Nabil & Reguig, 2015; Peltola, 2012). Similarly, with the degree zero method, when this method is used on large sections of artifacts, it results in slope-like shapes on the R-R interval tachogram (Nabil & Reguig, 2015; Peltola, 2012). This causes false trends and biases in LF ms^2 and VLF ms^2 (Nabil & Reguig, 2015; Peltola, 2012). The c*ubic interpolation* uses four data points to calculate the polynomial (Nabil & Reguig, 2015; Peltola, 2012). This method is considered a non-linear analysis, examining the complexity and irregularity of R-R interval time series (Nabil & Reguig, 2015; Peltola, 2012). Therefore, the presence of a falsely correlated signal may cause problems in obtaining accurate HR measures, especially if there is high number of artifacts, while it does not result in flat section in R-R interval tachogram (Nabil & Reguig, 2015; Peltola, 2012). Finally, in the *cubic spline interpolation*, corrected values are calculated using numerous data points by placing a third-degree polynomial (Nabil & Reguig, 2015; Peltola, 2012). Similarly, as with cubic interpolation, spline interpolation may cause biases in the R-R interval time series if the number of artifacts is high (Nabil & Reguig, 2015; Peltola, 2012). Technical details of these correction methods can be found in (Nabil & Reguig, 2015) study.

Giles et al., (2017) assessed the accuracy of deletion, interpolation methods, and Kubios (ver 2.2) in healthy individuals during incremental exercise with intensities varying from <40% to 80-100% of VO_{2max}. They found that degree one (linear interpolation) resulted in the smallest bias and effect size (ES) in majority of HRV measures compared to deletion, interpolation methods, and Kubios HRV (ver 2.2). However, they reported that biases and ESs at exercise intensities >

60% of VO_{2max} were large for RMSSD, LF/HF ratio, SD1, and Sample Entropy despite of correction methods. Nevertheless, a consensus regarding the best manual correction method is still lacking in the current literature while most studies agree that deletion should not be used for artifact correction.

2.5.3. Automatic Correction

While the MC is more reliable method than automatic correction of artifacts, manual correction is tedious and requires a long time of careful R-R interval editing and certain level of expertise. For this reason, various HRV software packages with automatic correction options were developed to conveniently edit R-R intervals, which may perform sufficiently in healthy adults with small number of artifacts (Peltola et al., 2012).

These software packages are not only used for automatically correcting artifacts but also for calculating HRV measures derived from R-R intervals. Some of the software packages are device-independent suggesting that they are not bundled with ECGs and HR monitors and that R-R intervals are downloaded from both devices and uploaded to the software to calculate the HRV measures. Thus, correction of artifacts from both devices can either be performed manually independent of the software or automatically with the software, but the calculation of HRV measures derived from manually or automatically corrected R-R intervals of ECG and HR monitor are carried out with the same software. Other software packages are device-dependent indicating that they come bundled with ECGs (e.g., CardioPerfect WorkStation) and HR monitors (e.g., Polar ProTrainer 5^{TM}) and that artifacts can only be automatically corrected and HRV measures derived from automatically corrected R-R intervals of ECG and HR monitor are calculated separately by each devices' individual software package.

The most used HRV software package for editing artifacts is Kubios HRV (The Biomedical Signals Analysis and Medical Imaging Group, University of Kuopio, Finland) with citations in about 1000 studies (Kubios HRV [ver. 3.2] User's Guide, 2019). Kubios started producing commercial (Premium) as well as free (Standard) software packages since January 2017. The commercial version began with Kubios HRV 3.0 and it was updated in January 2019 with Kubios HRV 3.2, which can be reached at the following reference (Kubios HRV [ver. 3.2] User's Guide, 2019). While the Standard version is primarily designed for non-commercial research and personal use, the Premium version is produced for researchers. Unlike the Standard, the Premium version calculates all time, frequency, and non-linear measures in addition to presenting time varying analysis. The Premium version also supports a broad range of ECG and HR in addition to photoplethysmogram data, whereas the Standard version supports only the data from HR monitors. The further details of the differences between Standard and Premium versions of Kubios HRV 3.2 are present in the following reference (Kubios HRV [ver. 3.2] User's Guide, 2019).

Kubios integrates visualizations of the R-R interval time series (i.e., tachogram) via a graphical interface and offers two artifact correction options including 1) AC; and 2) TBC. The AC option is available only in Kubios HRV Premium. The AC contains a robust algorithm for detecting artifacts with technical and physiological origins, which was validated by comparing it to Massachusetts Institute of Technology-Beth Israel Hospital arrhythmia database, demonstrating 97% and 99.9% of successes in correctly detecting artifacts and normal beats, respectively (86, 87). The technical details of the AC algorithm for detecting and correction artifacts are presented in the following reference (Kubios HRV [ver. 3.2] User's Guide, 2019).

The TBC detects and corrects the artifacts if they are outside of the thresholds including very low (0.45 sec), low (0.35 sec), medium (0.25 sec), strong (0.15 sec), and very strong (0.05

sec) compared to a local R-R interval average. The correction threshold can also be customized if the magnitude of difference does not include these thresholds. Each subjects' data should be individually treated when using the TBC due to the high inter-individual variations observed in HRV data. Thus, using the same threshold for every subject can overcorrect the R-R intervals, and consequently, introduce considerable bias into HRV measures. *In addition, the lowest correction method should always be a priority when using the TBC method to prevent potential overcorrections of normal R-R intervals* (Kubios HRV [ver. 3.2] User's Guide, 2019).

Figure 10 shows the R-R intervals with artifacts including: A) T3 at 00.00.05; and B) T4 at 00.00.15 and T5 at 00.00.27. Corrections of these artifacts can be applied using the AC or TBC methods under the *RR Interval Time Series Options* of the user interface. Whether or not a correction method affects normal R-R intervals can be seen by checking the plot on the R-R data axis, showing the total number and percent of artifacts. Plotted artifacts are replaced with R-R intervals created by the cubic spline interpolation. In addition to automatic artifact correction, Kubios offers a manual correction option if ECG data are available. Artifacts can be corrected by fixing the falsely detected R-waves: all R-waves on the R-R tachogram are marked with a "+" sign (Figure 11). The falsely plotted signs can be adjusted by right clicking and selecting "Move". If moving the signs does not correct the artifact, then it can be removed by right clicking and selecting "Remove" or a new sign (R-peak) can be added by right clicking and selecting "Add". The details of the data pre-processing can be found in the following reference (Kubios HRV [ver. 3.2] User's Guide, 2019).

Figure 10. Visual demonstration of Kubios HRV Premium (ver 3.2) corrections of T3, T4, and T5 artifacts on the R-R tachogram

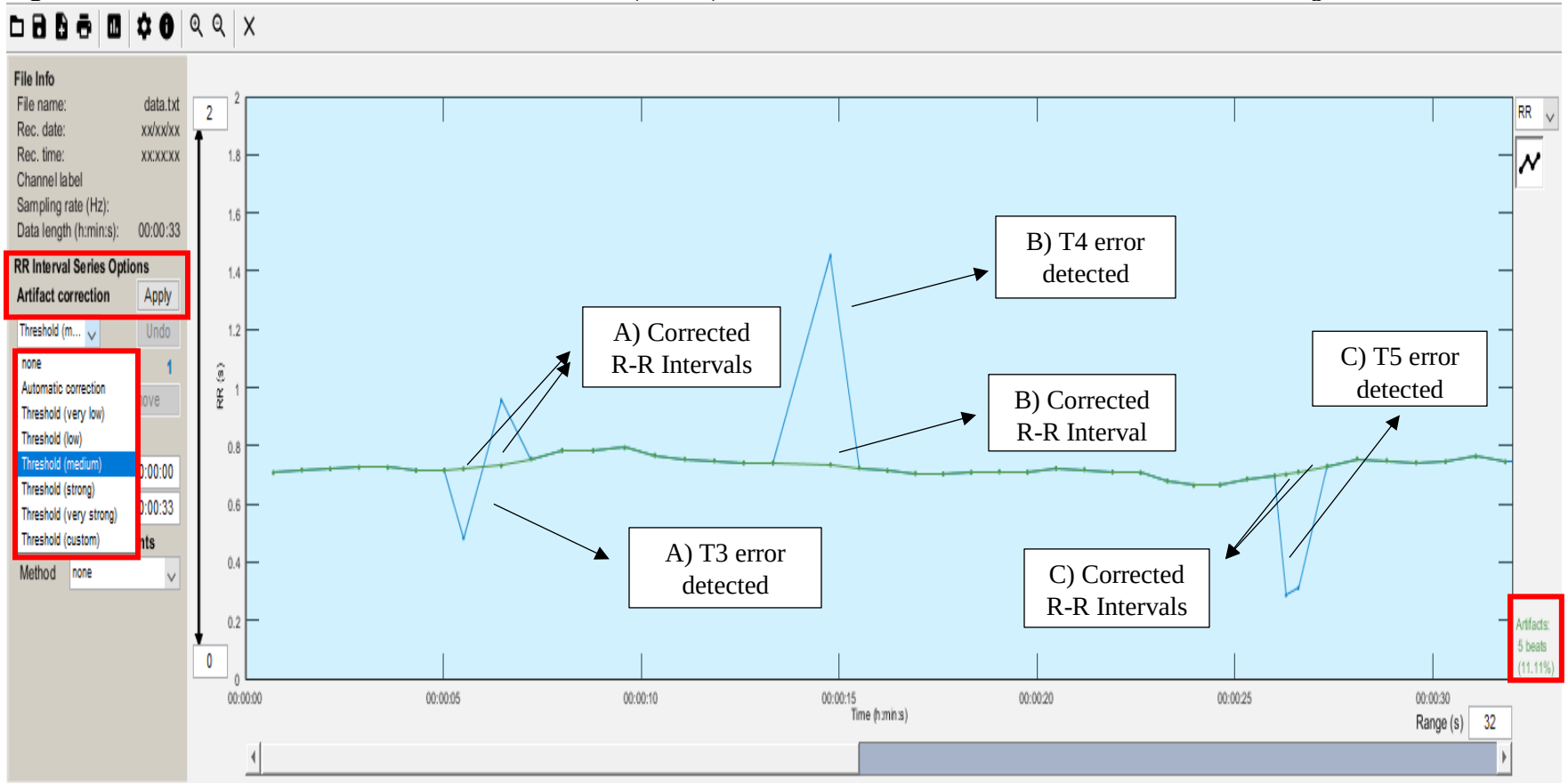
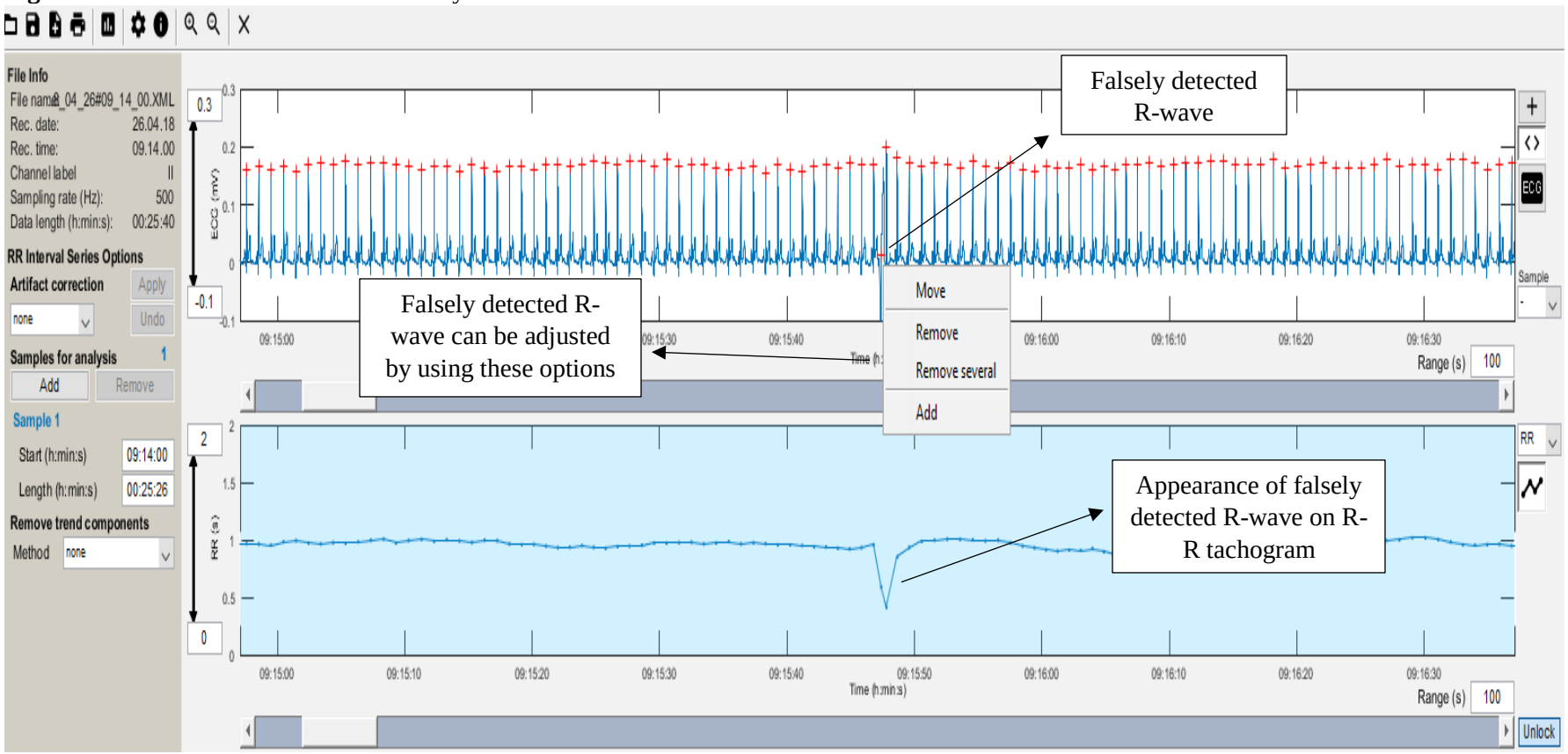


Figure 11. Visual demonstration of a falsely detected R-wave and its manual correction



2.6. Validation of HRV Monitors

Accuracy of HR monitors including the Polar S810^{TM} (Gamelin et al., 2006; Gamelin et al., 2008; Kingsley et al., 2005), Polar S810i^{TM} (Vanderlei et al., 2008), RS800G3^{TM} (De Rezende Barbosa et al., 2016) , RS800CX^{TM} (Vasconcellos et al., 2015), and V800^{TM} (Giles et al., 2016) have been compared to the gold standard ECG with 12-lead, 5-lead, 3-lead, and 2-lead in the supine and standing positions as well as during exercise (Caminal et al., 2018; Giles et al., 2017). However, these studies seem to have produced different results depending on the HR monitor, study setting, artifact correction methods, software package for HRV analysis, and subject characteristics (e.g., age, body mass index [BMI], gender, VO_{2max}). Further, the most obvious difference between the studies was the number of HRV measures, particularly the absence of reporting non-linear measures.

Error rate in total number of R-R intervals varied depending on HR monitor model. In the supine position, it appears that the latest version of Polar HR monitor V800^{TM} produced the lowest error rate. For example; Giles et al., (2016) and Giles et al., (2017) that validated Polar V800^{TM} reported error rates of 0.08% and 0.10% among healthy individuals, whereas Kingsley et al., (2005), Gamelin et al., (2006), and Vanderlei et al., (2008) who examined the validity of Polar S810^{TM} showed error rates of 0.32%, 0.40%, and 6.93% among healthy individuals (Board et al., 2016). Also, it has been shown that Polar V800^{TM} produced error rates proportional to the exercise intensity among healthy individuals, which could be the result of increased chest movement during exercise. Gamelin et al., (2017) who validated the Polar V800^{TM} among 18 healthy individuals during exercise performed under four intensities (<40%, 40-60%, 60-80%, 80%-100% of VO_{2max}) reported error rates of 0.90% at <40% of VO_{2max}, 2.25% at 40-60% of $VO_{2max,}$ 3.29% at 60-80% of $VO_{2max,}$ and 4.46% at 80-100% of VO_{2max}. In addition, Caminal et al., (2018) that validated Polar

V800™ among 22 healthy individuals during a running of six mountain routes reported an error rate of 0.71%, which is expectedly higher than those reported at rest.

Current literature of validations studies show that using an identical correction method for pre-processing (i.e., detection of R-R intervals and correction of artifacts) and software package for subsequent HRV analyses improves agreeability and interchangeability of the R-R intervals and HRV measures between HR monitors and simultaneously recorded ECGs.

Agreeability between the corrected R-R intervals of Polar V800™, RS800CX™, and S810™ HR monitors and ECGs were high in the supine position. For example; (Giles et al., 2016) showed bias (i.e., mean difference between two methods of measurement) of 0.06 and limits of agreement (LoA [i.e., the mean bias ± 1.96 x SD, the total error between two method of measurement]) ranges of -4.33 to + 4.45 ms between the corrected R-R intervals of Polar V800™ and the ECG among 20 healthy individuals (Board et al., 2016). Similarly, Vasconcellos et al., (2015) demonstrated bias of -10 and LoA ranges of -51.1 to 31.0 ms among 15 adolescents with obesity; and Montano et al., (2016) reported bias of +10 and LoA ranges of 8.0 to 12.0 ms between the corrected R-R intervals of Polar RS800CX™ and the ECG among 20 healthy individuals (Board et al., 2016). Additionally, between the corrected R-R intervals of Polar S810™ and the ECG, (Kingsley et al., 2005) reported bias of -0.06 and LoA ranges of -3.04 to +3.04 ms among 8 healthy individuals; Gamelin et al., (2006) showed bias of 0.9 and LoA ranges of -11.0 to +13.0 ms among healthy and physically active individuals; and Porto et al., (2009) reported bias of -1.85 and LoA ranges of -6.37 to 2.67 ms among 25 healthy and 8 individuals with obesity (n=2), emotional exhaustion (n=1), mitral valve prolapse (n=2), bicuspid aortic valve stenosis (n=1), Chagas' disease (n=1), and Asthma (n=1) (Board et al., 2016).

Agreeability of the R-R intervals between Polar V800™ and S810™ HR monitors and ECGs were also high in the standing position. In the standing position, Giles et al., (2016) reported bias of 0.59 and LoA ranges of -1.70 to +2.87 ms between the corrected R-R intervals of Polar V800™ and the ECG among healthy individuals. Moreover, between the corrected R-R intervals of Polar S810™ and the ECG, (Gamelin et al., 2006) showed bias of -0.70 and LoA ranges of -3.89 to 2.50 ms among healthy and physically active individuals and Porto et al., (2009) reported bias of 1.0 and LoA ranges of -6.0 to 8.5 ms among healthy and clinical populations (Board et al., 2016).

While Giles et al., (2017) did not report bias and LoA information of R-R intervals, Caminal et al., (2018) showed bias of <1 ms and LoA ranges of -3.55 to +3.57 ms among health individuals during exercise. (Kingsley et al., 2005) validated the Polar S810™ during exercise performed under the same exercise intensities as those reported by Giles et al., (2017). They did not report biases for varying exercise intensities but showed that the agreeability decreased as the exercise intensity increased with LoA ranges varying from -6.79 to 6.75 ms at <40% to -9.16 to 9.10 ms at 80-100% of VO_{2max} (Board et al., 2016).

Furthermore, interchangeability between the corrected R-R intervals of Polar V800™, RS800CX™, and S810™ HR monitors and ECGs was excellent in the supine position. For instance, it has been shown that intra-class correlations [(ICCs), defined as poor when ICC<0.50, moderate when ICC was between $\geq$0.50 and <0.75, good when ICC was between $\geq$0.75 and <0.90, and excellent when ICC was $\geq$0.90 (Koo & Li, 2016)], were 1.00 for Polar V800™ (Giles & Draper, 2018; Giles et al., 2016), 0.98 (Vasconcellos at al., 2015) and 0.99 (Montano et al., 2016) for RS800CX™ , and 0.99 (Gamelin et al., 2006) and 1.00 (Kingsley et al., 2005) for S810™ (Board et al., 2016). Moreover, in the standing position, it has been demonstrated that ICCs were 1.00 for V800™ (Giles et al., 2017; Giles et al., 2016) and 0.99 for S810™ (Gamelin et al., 2006), indicating

an excellent interchangeability between the HR monitors and ECGs (Board et al., 2016). While Caminal et al., (2018) and Giles et al., 2017) did not report ICCs during exercise, Kingsley et al., (2005) showed that the interchangeability decreased as the exercise intensity increased with ICCs ranging from 1.00 at <40% to 0.93 at 80-100% of VO_{2max}.

A good agreeability has been confirmed for all time, frequency, and non-linear measures with small biases and tight LoA ranges calculated from corrected R-R intervals recorded by Polar S810™, RS800CX™ ,RS800G3™ and V800™ in supine position and standing position. However, RMSSD with a bias of -8.1 and LoA ranges of -10.4 to -5.8 ms, $LFms^2$ with bias of 28.0 and LoA ranges of 25.6 to 30.5 ms^2, and $HFms^2$ with bias of -228.0 and LoA ranges of -230.7 to -225.4 ms (Montano et al., 2016); RMSSD with bias of 13.8 and LoA ranges of -18 to 45.6 ms, pNN50% with bias of 5.6 and LoA ranges of -15.0 to +26.2 %, LFnu with bias of -7.5 and LoA ranges of -34.7 to +19.7, and HFnu with bias of 7.6 and LoA ranges of -19.4 to +34.6 for Polar RS800CX™ (Vasconcellos at al., 2015); and $LFms^2$ with bias of 36.3 and LoA ranges of -42.3 to +114.9 ms^2 and HF ms^2 with bias of 27.3 and LoA ranges of -137.5 to 192.1 ms^2 for Polar RS800G3™ (De Rezende Barbosa et al., 2016) showed that the agreeability was poor for these variables between the Polar RS800CX™ and RS800G3™ HR monitors and ECGs in supine position.

Kingsley et al., (2005) that examined the validity of Polar S810™ during exercise bouts performed at <40%, 40-60%, 60-80%, and 80-100% of VO_{2max} reported high agreeability for LF ms^2 and HF ms^2. However, Giles et al., (2017) that compared various correction methods including Kubios HRV (ver 2.2), deletion, linear interpolation, cubic interpolation, cubic spline interpolation, and degree zero during exercise bouts at the same intensities reported by Kingsley et al., (2005) showed poor agreeability for RMSSD, LF/HF, SD1, and Sample Entropy at exercise intensities > 60% of VO_{2max}. Also, Camelin et al., (2018) that validated Polar V800™ among healthy individuals

who performed six mountain slopes reported a high agreeability with biases <1 and tight LoA ranges for all HRV measures.

An excellent interchangeability has been confirmed for time, frequency and non-linear measures calculated form R-R intervals recorded by Polar V800™ and ECG with ICCs ranging from 0.98 and 1.00 for all other measures in supine and standing positions (Giles et al., 2016). On the contrary, Nunan et al., (2008) that examined the validity of Polar S810™ among 33 healthy individuals showed poor interchangeability for LF ms^2 with an ICC of 0.70, HF ms^2 with an ICC of 0.65, LF nu with an ICC of 0.51, HF nu with an ICC of 0.62, and LF/HF with an ICC of 0.76. Similarly, Vasconcellos et al., (2015) reported that Polar RS800CX™ produced poor interchangeability for pNN50% with an ICC of 0.47, LF nu with an ICC of 0.31, and HF nu with an ICC of 0.32. In addition, De Rezende et al., 2016 reported that Polar RS800G3™ resulted in poor interchangeability for LF nu and HF nu with ICCs of 0.74 for both variables (Board et al., 2016).

Giles et al., (2017) showed that Polar V800™ produced poor interchangeability for LF ms^2 with an ICC of 0.67 and HF ms^2 with an ICC of 0.68 during an exercise bout performed at 40-60% of VO_{2max}; VLF ms^2 with an ICC of 0.62, LF ms2 with ICC of 0.66, and LF/HF with an ICC of 0.56 during an exercise bout performed at 60-80% of VO_{2max}; and HF ms^2 with an ICC of 0.51 during an exercise bout performed at 80-100% of VO_{2max}. Giles et al., (2017), however, reported an excellent agreement for all HRV measures during exercise bouts performed <60% of VO_{2max}. Furthermore, Caminal et al., (2018) reported that Polar V800™ resulted in excellent interchangeability for all HRV measures.

The use of different correction methods for pre-processing of R-R intervals and software packages can increase the potential for errors. Calculating the HRV measures derived from

automatically corrected R-R intervals with separate device-dependent software packages results in incomparable HRV measures the supine position. For example; Wallen et al., (2012) performed correction of artifacts of the Polar RS800CX™ HR monitor and ECG and subsequent calculations of HRV measures with individual Polar Pro Trainer (ver. 5.0) and CardioPerfect software packages among a clinical population with varying level of emotional exhaustion. Although Wallen et al., (2012) confirmed the interchangeability for all HRV measures derived from Polar RS800CX™ in place of those derived from ECG for men with an ICC of 0.8, they did not confirm the interchangeability for women over 60 years with an ICC <0.75 on any HRV measures. In addition, it has been shown that the Polar RS800CX™ produced large biases and LoA ranges for all HRV measures, suggesting low agreeability between both devices among clinical populations (Wallen et al., 2012). Due to the low agreeability and interchangeability results between Polar HR monitors and ECGs, the Polar stopped providing device-dependent software packages since December 2015.

The current evidence indicates that accuracy of the Polar V800™ HR monitor improved compared to the previous versions of Polar S810™ and RS800CX™. Also, generally high biases and LoA ranges and low ICCs of the Polar S810™ and RS800CX™ appear to result mainly from the use of device-dependent Polar software package for the calculation of HRV measures. Therefore, using an identical device independent HRV software package can prevent the potential bias into HRV measures derived from HR monitors in clinical populations such as patients with hypertension. Overall, HR monitors in sports and exercise settings show that SDNN, RMSSD, and LF/HF ratio consistently have good to excellent interchangeability with ECGs, but this information is lacking in clinical populations due to the scarcity of research in these individuals.

In conclusion, researchers and clinicians have become increasingly interested in using HR monitors due their convenience in measuring HRV, a biological marker that is strongly correlated

with compromised parasympathetic modulation, a common finding among individuals with hypertension. HRV measures, however, can be substantially distorted by artifacts with technical and physiological origins when using HR monitors that can lead to errors in interpreting the current status of hypertension. Therefore, if possible, the segment of R-R intervals free of artifacts should be selected as a first option. However, if that is not possible and the error rate of a sample >10% then artifacts should be corrected with a proper method. The MC is the most accurate and reliable method in correcting artifacts compared to the software packages offering automatic correction methods. The Polar Pro-trainer (ver.5), a device-dependent software package produce incomparable HRV measures among healthy and clinical populations due to the failure of correcting artifacts properly. Hence, Kubios HRV Premium (ver 3.2), a device-independent software package may be better in correcting artifact more accurately and thus produce comparable measures among healthy individuals at rest. Since the AC method was not available with the previous versions of Kubios HRV, the literature does not provide information as to which method of two can be chosen in correcting artifacts. Of note, however, if an error rate of a sample is >20% the data may result in inaccurate and unreliable HRV measures regardless of the correction methods. Additionally, deletion method should be used for correcting artifacts under any circumstances since it introduces substantial bias into HRV measures. Finally, improving the algorithms of HR monitors in detecting R-R intervals and the materials (e.g., the chest strap) may reduce the dependence on the artifact correction methods, which is what makes ECG the gold standard in measuring HRV.

CHAPTER 3: METHODS

3.1. Study Overview

All subjects attended one laboratory session between 6:00 and 11:00 am. Prior to the resting HRV measurement, subjects' chests were cleaned for the ECG attachment and 12-lead ECG electrodes were placed on subjects. Later, the chest strap (H7) of Polar V800^{TM} HR monitor was placed just below the pectoralis major muscles. Then, resting HRV was measured for 5 min in supine position following 5 min of resting period in the same position. Subjects were instructed to relax as much as possible but not sleep, move or talk. The R-R interval time series from ECG was stored in CASE^{TM} (ver 6.6) GE Healthcare system while those from Polar V800^{TM} HR monitor was automatically stored in Polar Flow web service. Subjects completed an informed consent approved by the Institutional Review Boards of the University of Connecticut and Hartford Hospital. Subjects were recruited on rolling basis, beginning October 2016 until study completion in May 2018.

3.2. Study Population

Subjects were recruited from the surrounding community with direct mailings and posting of flyers, media advertisements, social media, previous studies, and from places of work and college campuses with the posting of flyers, listservs, class announcements, and newsletters. Adults (n=25) with elevated (SBP ≥120-<130 mm Hg; DPB < 80 mm Hg) to established hypertension (SBP >130 mm Hg; DBP >80 mm Hg) according to the updated American College of Cardiology/American Heart Association Guidelines for hypertension were enrolled (Arnett et al., 2019). Subjects who were being sedentary to regularly physically active (i.e., 150 min a week moderate or 75 min a week vigorous intensity exercise); were free of cardiovascular, pulmonary, renal, metabolic, or other chronic diseases and depression; did not smoke for at least 6 months prior to entry; and consumed less than two alcoholic drinks daily were included. Subjects with a medical history of

cancer-related lymphedema were excluded due to the risk of infection during HRV measurement. Moreover, subjects were excluded if they were trying to gain or lose weight because of the confounding effect of weight gain and loss on HRV (Karason et al., 1999). Furthermore, women who were pregnant, of planning to become pregnant, or were lactating were excluded (Stein et al., 1999).

3.3. Procedure

Subjects were informed to consume a light breakfast 2-3 hours before and abstain from drinks containing alcohol and caffeine at least 48 hr before the testing. BMI ($kg \cdot m^{-2}$) was measured from body weight and height collected with a calibrated balance beamscale, and waist circumference (WC) was measured using a Gullick tape at the narrowest part of the torso (Pescatello, 2013). Testing BP was measured following the standard American Heart Association procedures with an automated BPTRU monitor (BPTRU Medical Devices; Coquitlam, Canada). The measurements of BMI, WC, and BP preceded HRV recording that was performed in a supine position in a quiet, low-light, and temperature-controlled room.

Subjects' chests were shaved if necessary. The ECG electrodes were placed in the Mason-Likar configuration using the GE Stress System (CASE, Milwaukee, WI) (Papouchado et al., 1987). The ECG signal was checked to ensure that it was consistent and free of noise. After the signal was confirmed to be acceptable, the Polar V800^{TM} H7 chest strap was fitted below the pectoralis major muscles and applied as described by the manufacturer. Subjects were placed in supine position for 10 min, but the last 5 min of which was recorded for the analysis. During the recording, subjects were asked not to move, sleep or talk, and their breathing frequency was paced at 12 breaths/min^{-1} using a metronome to control for the respiratory influences on HRV.

3.4. R-R Interval Recording

The 12-lead ECG and Polar V800^{TM} HR monitor with Polar H7 chest strap were started to simultaneously record R-R intervals at a sampling frequency of 1000 Hz. Cardiology XML files obtained from ECG were imported into Kubios HRV Premium (ver 3.2) (The Biomedical Signals Analysis and Medical Imaging Group, University of Kuopio, Finland) in order to export the R-R intervals detected automatically by its built-in QRS detection algorithm. Kubios HRV Premium (ver 3.2) marked each R-wave with "+" sign that can be moved or removed to correct falsely detected R-waves. The detected R-R intervals were manually inspected on the ECG tachogram to ensure that there were no false R-wave detections. If an R-wave was falsely determined, it was replaced by moving "+" signs on the correct R-wave. Then the detected R-R intervals from ECG were saved in a space delimited ASCII text file. In addition, the R-R intervals recorded by Polar V800^{TM} HR monitor were exported from the Polar Flow web service (Version 2.3; Polar Electro Oy, Kempele, Finland) in a space delimited ASCII text file.

3.4. Pre-processing of R-R Intervals

3.4.1. Artifact identification

First, the R-R intervals from ECG and Polar V800^{TM} HR monitor in the space delimited ASCII text files were imported into the same spreadsheet side-by-side. Next the R-R intervals from both devices were synchronized by inserting 0 ms ensuring the comparison between the ECG and UN V800^{TM} HR monitor R-R intervals. The technical artifacts (i.e., missed beasts) from the Polar V800^{TM} HR monitor recordings or physiological artifacts (i.e., non-sinus beats) were then identified by comparing the R-R intervals from both devices. An artifact from the V800^{TM} HR monitor was identified when the differences between ECG and Polar V800^{TM} HR monitor R-R intervals were greater than 20 ms. Subsequently, the differences between the R-R intervals of ECG and Polar

V800^{TM} HR monitor (i.e., type of artifacts) were assigned one of seven error types of the error identification and correction guideline developed by Gamelin et al., (2006) and Gamelin et al,m (2008) and recently updated by Giles et al., (2016). The updated version of the guideline contains extra two artifacts including T6-a and T6-b that were found with Polar V800^{TM} HR monitor. The seven types of error are described under the heading of *2.4.1. Types of Artifacts in R-R* Intervals in Chapter 2 (pg. 35-42). We followed the updated guidelines and reported all seven types of artifacts from T1 to T6b.

3.4.2. Artifact Correction

The artifacts were manually and automatically corrected after their identification. For the MC, non-sinus beats in both signals (N=3) were replaced by interpolated R-R intervals from adjacent R-R intervals. Corrections were made only for T2, T3, T4, T5, and T6b since it is not possible to identify T1 and T6b artifacts without simultaneous ECG recordings. Methods of correction for the errors are described under the heading of *2.5.1. Manual Correction* in Chapter 2 (pg.45-50).

The AC and TBC methods of Kubios HRV Premium (ver 3.2) were used to automatically correct the artifacts. It should be noted that the AC was not available in Kubios versions before the first commercial Kubios HRV Premium (ver 3.0) was released in January 2017. For the AC, "Automatic Correction" under "R-R Interval Series Option" was selected and then " Apply" button was selected to correct the artifacts. For the TBC, an appropriate threshold among very low (0.45 sec), low (0.35 sec), medium (0.25 sec), strong (0.15 sec), very strong (0.05 sec), and custom under "R-R Interval Series Option" was selected and then "Apply" button was selected to correct the artifacts. When the artifacts were corrected with the TBC, the lowest level of correction was chosen to prevent potential overcorrection. When artifacts were detected by both methods, they were automatically replaced through the cubic spline interpolation, a type of error correction. After the

artifact corrections were made, the R-R interval time series was then considered normal and defined as N-N intervals (Kubios HRV [ver. 3.2] User's Guide, 2019).

3.4.2. Calculation of HRV Measures

Following the identification and correction of the artifacts, a corresponding segment of the N-N intervals with varying recording length in the ASCII text files from ECG and Polar V800^{TM} HR monitor was selected for the calculation of the HRV measures. Commercial Kubios HRV Premium (ver 3.2) analyzed the selected segments to obtain the time, frequency, and non-linear domains of HRV measures.

The time domain measures quantify the amount of variability within the sample and they included SDNN, RMMSD, and pNN50%. Frequency domain measures show the contribution of SNS and PNS modulation within the sample they included LF ms^2 HF ms^2, LFnu, HFnu, and LF/HF ratio. Power spectral density analysis reveals the content of signal's power (variance) versus frequency and can be analyzed by the autoregressive and fast Fourier transform methods (Shafer 2017). A fast Fourier transformation was performed to quantify power spectrum density into the LF (0.04–0.15 Hz) and HF (0.15–0.40 Hz) frequency bands. In addition, normalized units of the LF and HF bands and LF/HF ratio were calculated. Non-linear domain measures represent the unpredictable heartbeat dynamics caused by the complex interactions between a number of regulatory systems and they included SD1, SD2, and SD2/SD1. The non-linear measures were analyzed as a Poincaré plot, a type of graph where each N-N interval is plotted against next N-N interval, making a scatter plot (Shafer et al., 2017). The analysis consisted of placing an ellipse to the plotted points (Shaffer et al., 2017). The SD of each plotted N-N interval from the y= x axis (SD1 or ellipse's width), the SD of each plotted N-N interval from the y = x + average N-N interval (SD2 or ellipse's length), and SD2/SD1 ratio were then calculated (Shafer et al., 2017).

3.5. Statistical Analysis

The magnitude of difference between the R-R intervals and HRV measures from ECG and Polar V800^{TM} HR monitor were calculated by measuring effect size (ES) as the mean difference over the standard deviation of the difference (Thomas et al., 2010). The ES was defined as trivial when ES <0.2, small when ES was between $\geq$0.2 and <0.5, moderate when ES between $\geq$0.5 and <0.8, and large when $\geq$0.8 (Cohen 2013). The ICC with the 95% confidence interval (CI) assessed the concurrent validity (or interchangeable agreement) of the R-R intervals and HRV measures (Atkinson & Nevill, 1998). The ICC was defined as poor when ICC<0.50, moderate when ICC was between $\geq$0.50 and <0.75, good when ICC was between $\geq$0.75 and <0.90, and excellent when ICC was $\geq$0.90 (Koo and Li, 2016). Bland-Altman plots were created for the ECG R-R intervals versus Polar V800^{TM} HR monitor for the UN, AC, TBC, and MC R-R intervals. The 95% limits of agreement (LoA) for lower (-1.96) and upper limit (+1.96) were calculated as follows: 1) lower limit: mean difference - (SD of difference x 1.96); and 2) upper limit: (SD of difference x 1.96) + mean difference (Bland and Altman, 1986). Homoscedasticity and heteroscedasticity were inspected through a histogram and Q-Q plot. In the case that was detected in the heteroscedasticity in HRV measures, the data were logarithmically transformed before the calculation of LoA ranges. All statistical analyses were conducted using SPSS (Version 24; Chicago, IL, USA)

CHAPTER 4: RESULTS

4.1. Subject Characteristics

Participants were 17 men (68%) and 8 women (28%) between 18 and 55 years of age who were overweight to obese with hypertension (Table 4). Of these, six subjects were taking antihypertension medication that included diuretics (n=1), angiotensin II receptor antagonists (n=3), and β-Blockers (n=2).

Table 4. Subjects Characteristics (n=25, mean±SD)

Variable	**Results**
Age (year)	44.7±10.1
Height (cm)	172.3±11.0
Weight (kg)	93.7±30.6
Body Mass Index ($kg.m^{-2}$)	29.8±4.3
Waist Circumference (cm)	99.9±12.1
Heart Rate (bpm)	73.6±11.0
Systolic Blood Pressure (mmHg)	132.3±12.2
Diastolic Blood Pressure (mmHg)	84.3±10.2

4.2. Agreeability and Interchangeability of R-R Intervals

Table 5 presents the type, number, and percent of detected artifacts from the Polar V800^{TM} HR monitor among the total number of artifacts in the supine position. There were a total of 71 artifacts among 8325 total R-R intervals yielding an error rate of 0.85%, which was calculated by dividing the total number of artifacts by the total number or R-R intervals and multiplying the result by 100. The length of HRV measurement for eight subjects was less than 5 min due to the loss of connection between the HR monitor and the strap for unknown reasons. Therefore, the average length of the HRV measurement was 4.6±0.9 min and the number of R-R intervals was 333.0±70.5.

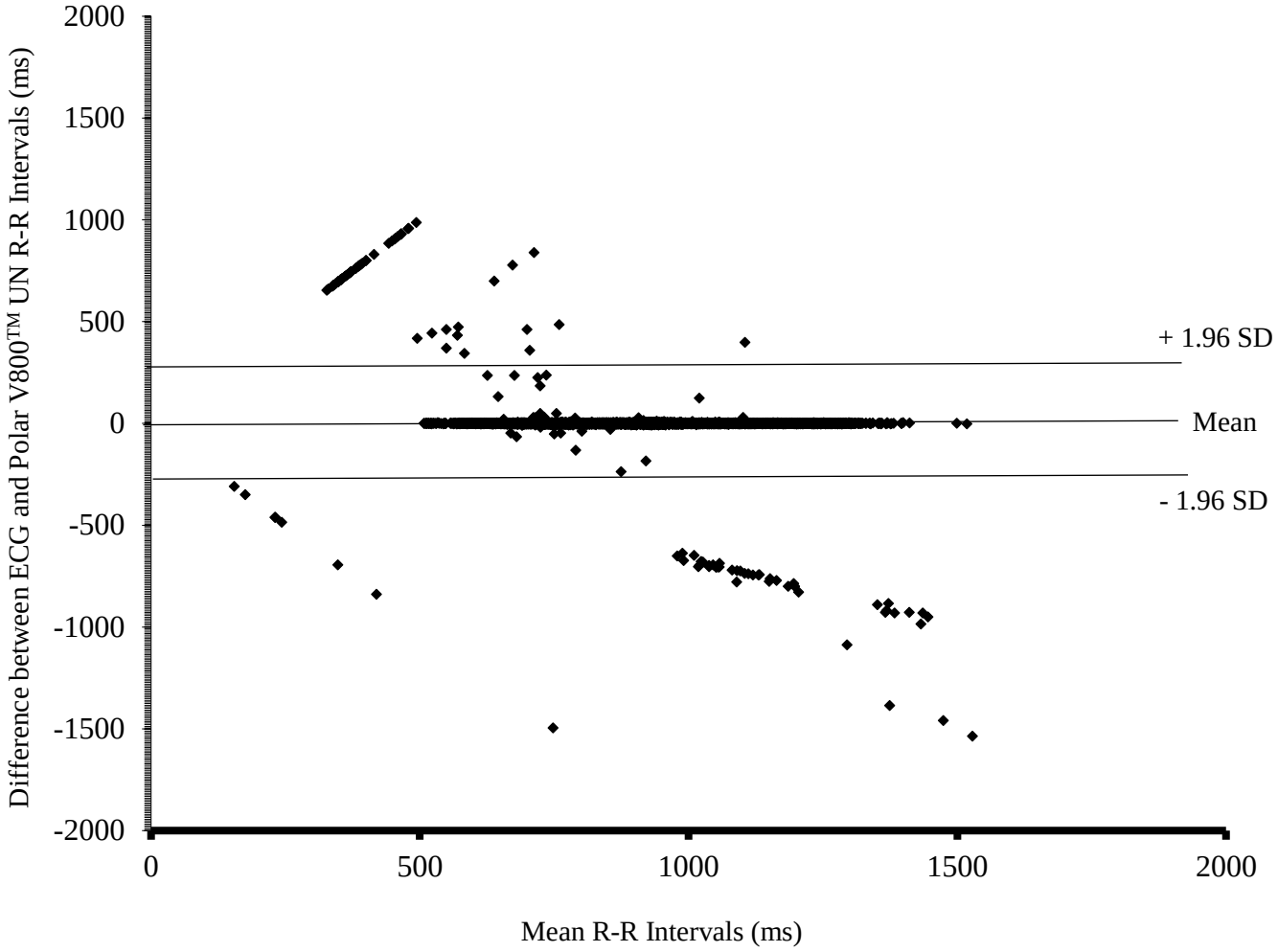
Figure 13 contains the Bland-Altman plots of the level of agreement and interchangeability between the R-R intervals from the ECG and the UN, AC, TBC, and MC R-R intervals from the Polar V800^{TM} HR monitor. The UN R-R intervals from the Polar V800^{TM} HR monitor were corrected using the AC and TBC methods of Kubios Premium (ver 3.2) and MC. While the bias (0.69 ms) and ES (0.004) were small, the UN R-R intervals resulted in the widest range of LoA (from -215.80 to 214.42 ms) (Figure 12). The AC method using the Kubios HRV Premium (ver 3.2) resulted in corrected R-R intervals with higher bias (3.79 ms), range of LoA (from -130.32 to 137.90 ms), and ES (0.024) (Figure 13) than the bias (1.16 ms), range of LoA (from -92.67 to 94.98 ms), and ES (0.008) of the TBC method (Figure 14). The MC method produced corrected R-R intervals with the smallest bias (0.37 ms), tightest range of LoA (-41.20 to 41.94 ms), and smallest effect size (0.002) (Figure 15). Furthermore, an improvement in the ICC of the UN R-R intervals occurred depending on the method of correction that was used. The ICC of UN R-R intervals went from 0.79 (95 % CI 0.78-0.80) to 0.91 (95 % CI 0.90-0.91) in the AC R-R intervals, 0.95 (95% CI 0.95-0.95) in the TBC R-R intervals, and 1.00 (95% CI 1.00-1.00) in MC R-R intervals.

Table 5. Types of errors detected in the Polar V800^{TM} HR monitor R-R intervals in supine position

Type of Error	**Number of Detected Errors**	**Percent of Detected Errors***
T1	5	7.0%
T2	1	1.4%
T3	5	7.0%
T4	49	69%
T5	7	9.9%
T6a	2	2.8%
T6b	2	2.8%

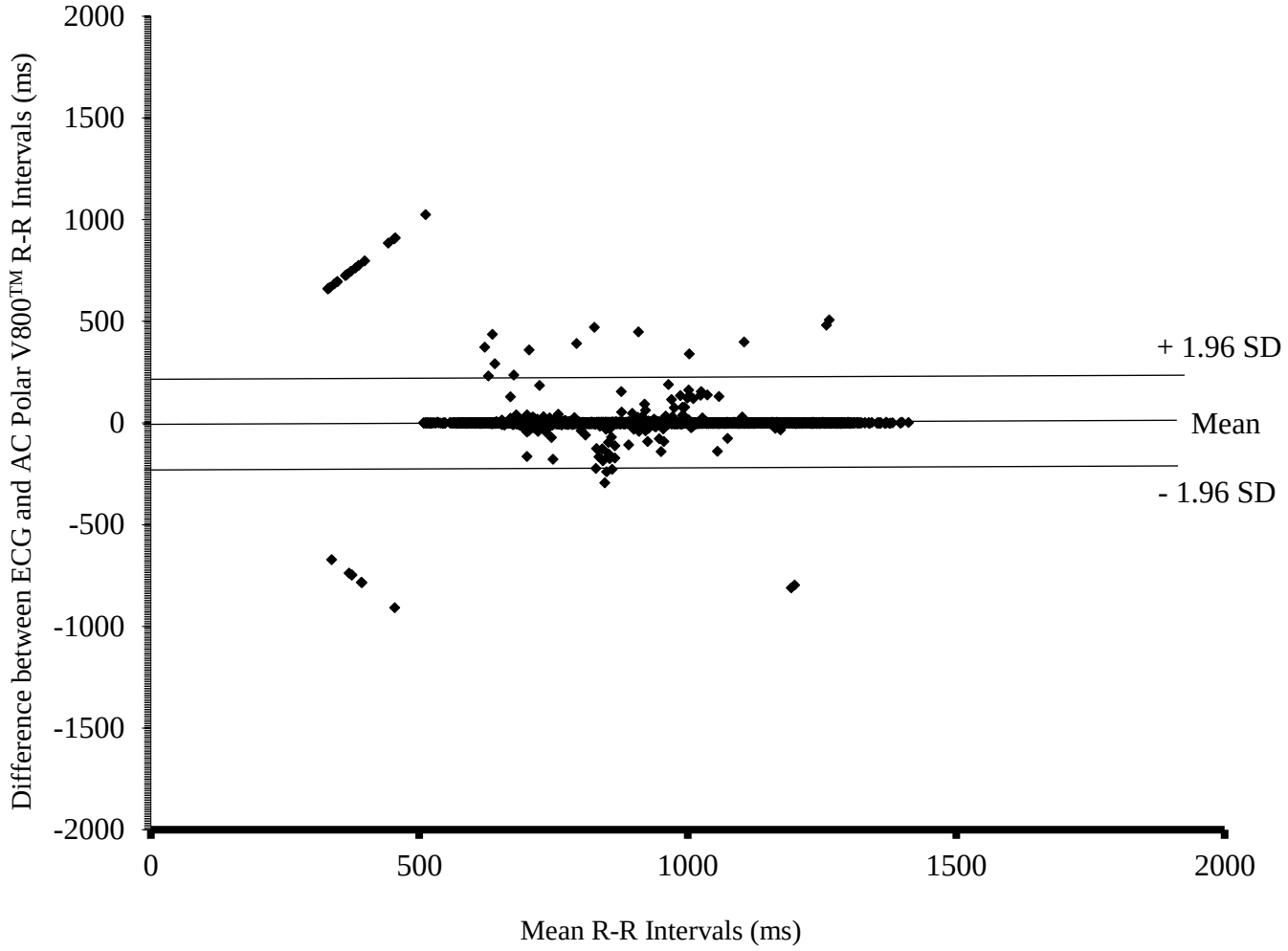
*Percent of detected errors is calculated as follows: (the number of a particular error/the total number artifacts) x 100. The total number of errors= 71.

Figure 12. Bland-Altman plots for the ECG and the UN Polar V800™ HR monitor R-R intervals



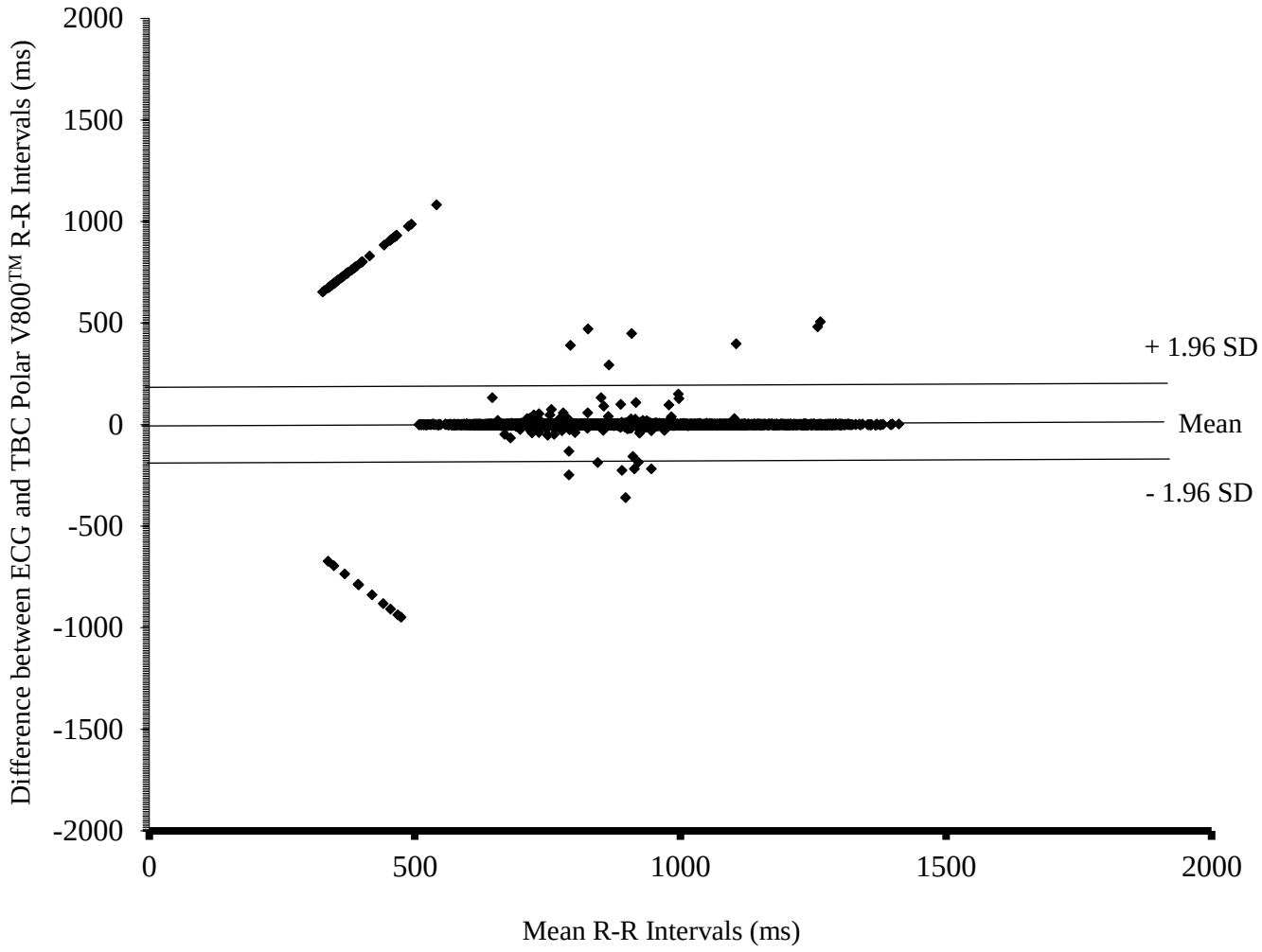
ECG: electrocardiogram; HR; heart rate; UN; uncorrected R-R intervals

Figure 13. Bland-Altman plots for the ECG and the AC Polar V800^{TM} HR monitor R-R intervals



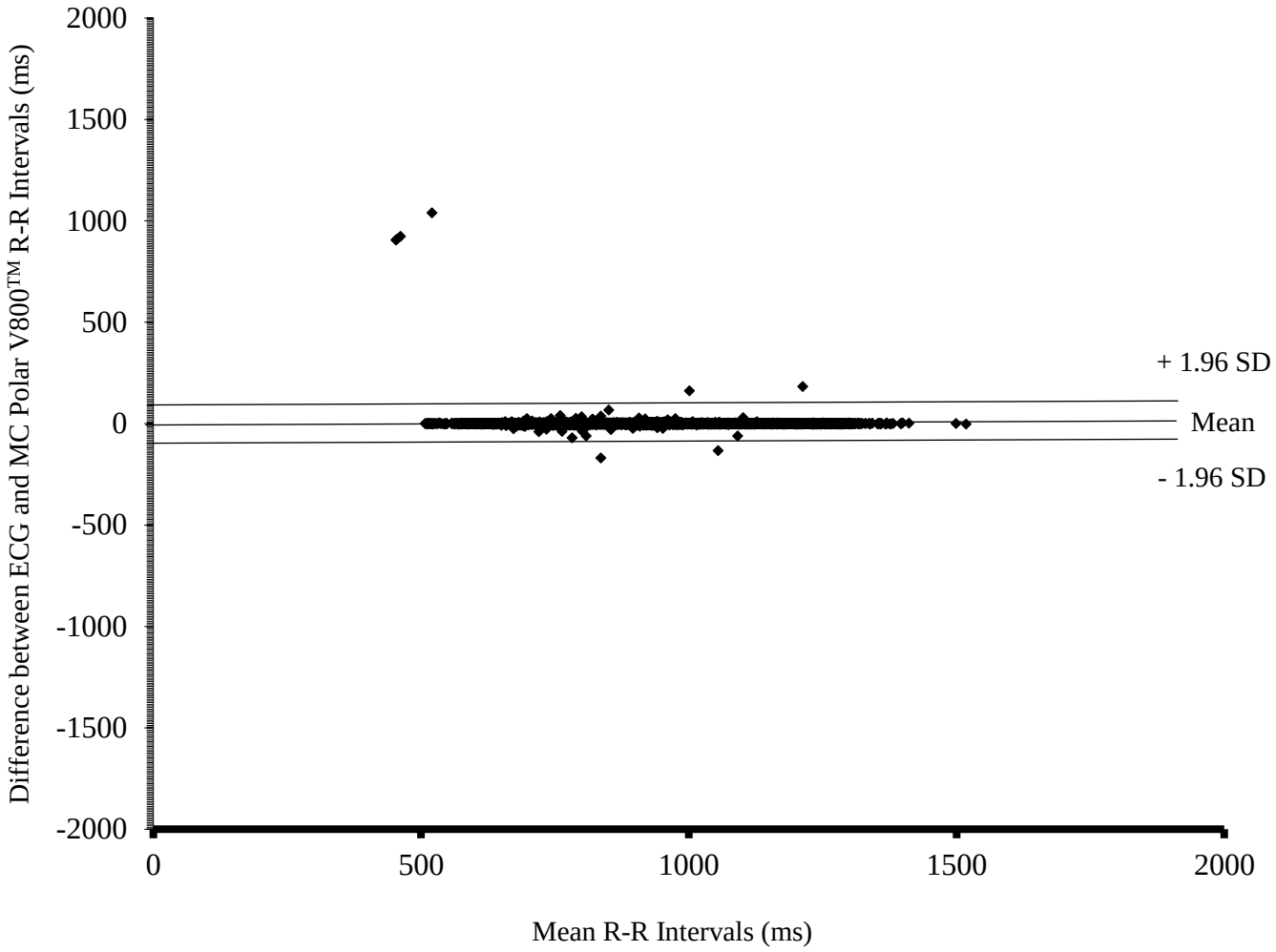
ECG: electrocardiogram; HR; heart rate; AC; R-R intervals corrected by automatic correction method of the Kubios HRV Premium (ver 3.2)

Figure 14. Bland-Altman plots for the ECG and the TBC Polar V800^{TM} HR monitor R-R intervals



ECG: electrocardiogram; HR; heart rate; AC; R-R intervals corrected by threshold-based correction method of the Kubios HRV Premium (ver 3.2)

Figure 15. Bland-Altman plots for the ECG and the MC Polar V800^{TM} HR monitor R-R intervals



ECG: electrocardiogram; HR; heart rate; AC; R-R intervals corrected manually

4.3. Agreeability and Interchangeability of HRV Measures

Table 6, 7, 8, and 9 contain the comparisons of the HRV measures calculated from UN, AC, TBC, and MC Polar V800^{TM} HR monitor R-R intervals versus those calculated from ECG R-R intervals using the Kubios Premium (ver 3.2) software.

The UN resulted in the largest biases and the widest LoA ranges for all HRV measures compared to the AC, TBC, and MC methods. The UN had ES ranging from 0.091 to 0.835 indicating the magnitude of difference between the HRV measures calculated from UN Polar V800^{TM} HR monitor and ECG R-R intervals was trivial to large and had 5 out of 11 HRV measures between 0.2≤ and <0.5, indicating moderate difference. Additionally, the UN had ICCs ranging from 0.070 to 0.98 suggesting poor to excellent interchangeable agreement but had 7 out of 11 HRV measures below <0.5, indicating poor interchangeable agreement (Table 6). Between the two correction methods of Kubios HRV Premium (ver 3.2), the TBC resulted in smaller biases than the AC for SDNN, RMSSD, pNN50%, LFnu, HFnu, LF/HF ratio, SD1, SD2, and SD2/SD1, but the AC produced smaller biases than TBC for LFms^2 and HFms^2. Additionally, when the TBC was compared to the AC method, the TBC produced tighter LoA ranges and smaller ES (<0.031 versus <0.085) for all HRV measures.

Moreover, the TBC method exhibited ICCs varying from 0.96 to 1.00, while the AC had ICCs ranging from 0.79 to 0.99, indicating that the interchangeability slightly improved from good-to-excellent to excellent when the correction was made with the TBC (Table 7 and Table 8). Whereas the MC produced the smallest biases for SDNN, RMSSD, pNN50%, LF/HF, SD1, SD2, and SD2/SD compared to the AC and TBC methods, the MC caused larger biases for LFms^2 and HFms^2 than the AC and also for LFms^2, LFnu, and HFnu than the TBC. Additionally, when the MC was compared to the AC and TBC methods, the MC resulted in the tightest LoA ranges and

smallest ES (<0.019) for all HRV measures. Furthermore, the MC had ICC of 1 for all HRV measures indicating excellent reliability for all HRV measures (Table 9).

The possible impact of gender, medication use (particularly β-Blocker use), VO_{2max}, BMI, and HRV measurement length was examined, but we found that they did not influence the biases, LoA ranges, ICCs, or ESs of the comparison of the HRV measures calculated from the UN, AC, TBC, and MC Polar V800^{TM} and ECG R-R intervals.

Table 6. Comparison of HRV measures calculated from UN Polar V800™ and ECG R-R intervals (mean ± SD)

HRV Measure	ECG (mean±SD)	Polar UN (mean±SD)	Bias (LoA)	ICC (95% CI)	Effect Size
SDNN (ms)	55.5±26.7	90.5±62.2	-34.95 (-143.58 to 73.69)	0.27 (-0.07-0.57)	0.730
RMSSD (ms)	38.3±29.4	92.8±96.3	-57.51 (-223.81 to 114.78)	0.21 (-0.11-0.52)	0.765
pNN50 (%)	13.6±16.2	15.1±15.9	-1.50 (-6.14 to 3.21)	0.98 (0.95-0.99)	0.091
LF (ms^2)	1215±1352.1	6924.2±16947.2	-5709.13 (-38536.30 to 27118.05)	0.53 (-0.97-0.57)	0.475
HF (ms^2)	740.8±1262.8	4861.0±6863.9	-4120.18 (-17136.40 to 8896.033)	0.07 (-0.21-0.39)	0.835
LF (nu)	67.3±18.7	59.7±20.3	7.53 (-30.13 to 45.20)	0.49 (0.14-0.73)	0.387
HF (nu)	32.7±18.6	40.2±20.2	-7.53 (-45.20 to 30.13)	0.49 (0.14-0.73)	0.387
LF/HF Ratio	3.6±4.0	3.0±4.2	0.67 (-3.37 to 4.71)	0.87 (0.72-0.94)	0.163
SD1 (ms)	27.1±20.8	65.7±68.2	-38.62 (-158.55 to 81.31)	0.21 (-0.11-0.52)	0.765
SD2 (ms)	72.7±33.6	106.5±62.1	-33.80 (-137.14 to 69.53)	0.37 (-0.01-0.66)	0.677
SD2/SD1 Ratio	3.4±1.4	2.7±1.6	0.66 (-1.45 to 2.76)	0.68 (0.32-0.85)	0.442

ECG: electrocardiogram; UN: uncorrected R-R intervals; ICC: intra-correlation coefficient; LoA: limits of agreement; SDNN: standard deviation of normal-to-normal N-N intervals; RMSSD: root mean square of the successive difference of intervals; pNN50%: the percentage of successive normal cardiac inter-beat intervals greater than 50 msec; LF: low frequency; HF: high frequency; SD: standard deviation.

Table 7. Comparison of HRV measures calculated from Kubios Premium (ver 3.2) AC Polar V800™ and ECG R-R intervals (mean ± SD)

HRV Measure	ECG (mean±SD)	Polar AC (mean±SD)	Bias (LoA)	ICC (95% CI)	Effect Size
SDNN (ms)	55.5±26.7	57.1±28.1	-1.60 (-23.27 to 20.06)	0.92 (0.83-0.96)	0.058
RMSSD (ms)	38.3±29.4	36.2±25.4	2.13 (-25.57 to 29.84)	0.87 (0.73-0.94)	0.078
pNN50 (%)	13.6±16.2	13.1±16.1	0.51 (-7.28 to 8.29)	0.97 (0.93-0.99)	0.031
LF (ms^2)	1215±1352.1	1214.2±1360.9	0.80 (-369.45 to 371.06)	0.99 (0.98-1.00)	0.001
HF (ms^2)	740.8±1262.8	749.1±1239.5	-8.27 (-1115.84 to 1099.293)	0.90 (0.79-0.96)	0.007
LF (nu)	67.3±18.7	66.7±19.6	0.57 (-24.29 to 25.44)	0.79 (0.57-0.90)	0.030
HF (nu)	32.7±18.6	33.3±19.6	-0.57 (-25.44 to 24.29)	0.79 (0.73-0.95)	0.030
LF/HF Ratio	3.6±4.0	3.5±3.9	0.12 (-1.76 to 2.00)	0.97 (0.94-0.99)	0.031
SD1 (ms)	27.1±20.8	25.6±18	1.51 (-18.12 to 21.14)	0.87 (0.73-0.94)	0.078
SD2 (ms)	72.7±33.6	75.6±36.4	-2.97 (-26.11 to 20.17)	0.94 (0.87-0.97)	0.085
SD2/SD1 Ratio	3.4±1.4	3.4±1.3	-0.07 (-1.01 to 0.87)	0.94 (0.86-0.97)	0.055

AC: R-R intervals corrected by automatic correction method of Kubios HRV Premium (ver 3.2)

Table 8. Comparison of HRV measures calculated from Kubios Premium (ver 3.2) TBC Polar V800™ and ECG R-R intervals (mean ± SD)

HRV Measure	ECG (mean±SD)	Polar TBC (mean±SD)	Bias (LoA)	ICC (95% CI)	Effect Size
SDNN (ms)	55.5±26.7	56.1±27.6	-0.55 (-10.59 to 9.49)	0.98 (0.96-0.99)	0.020
RMSSD (ms)	38.3±29.4	37.8±27.5	0.53 (-9.57 to 10.63)	0.98 (0.96-0.99)	0.019
pNN50 (%)	13.6±16.2	13.8±16.2	-0.16 (-1.33 to 1.02)	0.99 (0.98-1.00)	0.010
LF (ms^2)	1215±1352.1	1209.2±1352.5	5.86 (-275.86 to 287.59)	1.00 (0.99-1.00)	0.004
HF (ms^2)	740.8±1262.8	703.7±1201.6	37.07 (-460.59 to 534.74)	0.98 (0.95-0.99)	0.030
LF (nu)	67.3±18.7	67.3±18.0	-0.02 (-4.04 to 4.01)	0.99 (0.99-1.00)	0.001
HF (nu)	32.7±18.6	32.7±18.0	0.02 (-4.01 to 4.04)	0.99 (0.99-1.00)	0.001
LF/HF Ratio	3.6±4.0	3.5±3.9	0.12 (-0.79 to 1.02)	0.99 (0.98-1.00)	0.029
SD1 (ms)	27.1±20.8	26.7±19.5	0.38 (-6.78 to 7.53)	0.98 (0.96-0.99)	0.019
SD2 (ms)	72.7±33.6	73.8±35.7	-1.09 (-14.39 to 12.21)	0.98 (0.96-0.99)	0.031
SD2/SD1 Ratio	3.4±1.4	3.3±1.4	0.03 (-0.74 to 0.80)	0.96 (0.91-0.98)	0.021

TBC: R-R intervals corrected by threshold-based correction method of Kubios HRV Premium (ver 3.2)

Table 9. Comparison of HRV measures calculated from MC R-R intervals between the ECG and Polar (mean ± SD)

HRV Measure	ECG (mean±SD)	Polar MC (mean±SD)	Bias (LoA)	ICC (95% CI)	Effect Size
SDNN (ms)	55.5±26.7	55.3±26.7	0.21 (-0.70 to 1.12)	1.00 (1.00-1.00)	0.009
RMSSD (ms)	38.3±29.4	38.1±29.1	0.18 (-1.82 to 2.18)	1.00 (0.99-1.00)	0.006
pNN50 (%)	13.6±16.2	13.7±16.1	-0.11 (-1.52 to 1.30)	1.00 (0.99-1.00)	0.007
LF (ms^2)	1215±1352.1	1208.2±1351	6.85 (-65.77 to 79.48)	1.00 (1.00-1.00)	0.005
HF (ms^2)	740.8±1262.8	730.9±1267.1	9.94 (-44.20 to 64.07)	1.00 (1.00-1.00)	0.008
LF (nu)	67.3±18.7	67.6±18.6	-0.35 (-1.68 to 0.98)	1.00 (1.00-1.00)	0.019
HF (nu)	32.7±18.6	32.3±18.6	0.35 (-0.98 to 1.68)	1.00 (1.00-1.00)	0.019
LF/HF Ratio	3.6±4.0	3.7±4.0	-0.04 (-0.23 to 0.14)	1.00 (1.00-1.00)	0.011
SD1 (ms)	27.1±20.8	27.0±20.6	0.13 (-1.29 to 1.54)	1.00 (1.00-1.00)	0.006
SD2 (ms)	72.7±33.6	72.5±33.5	0.21 (-0.55 to 0.97)	1.00 (1.00-1.00)	0.006
SD2/SD1 Ratio	3.4±1.4	3.4±1.4	0.01 (-0.14 to 0.15)	1.00 (1.00-1.00)	0.005

MC: R-R intervals corrected manually

CHAPTER 5: DISCUSSION

In the current study, we compared raw R-R intervals and HRV measures derived from the Polar V800^{TM} HR monitor to the gold standard 12-lead ECG to determine their level of agreement and interchangeability among a sample of 17 men and 8 women with hypertension who were overweight to obese and of very poor to good cardiorespiratory fitness for men and women of their age. In addition, we sought to determine the level of accuracy of the MC as well as AC and TBC methods of Kubios HRV Premium (ver 3.2) in correcting artifacts among this sample. The results demonstrate that Polar V800^{TM} can provide R-R intervals consistent with the ECG and that HRV measures calculated from both devices are highly comparable in adults with hypertension in supine position as long as the raw R-R intervals are corrected.

The error rate in the current study (0.85%) was higher than those reported by Giles et al., (2016) (0.08%; -0.77%) and Giles et al., (2017) (0.10%; -0.75%) that validated the Polar V800^{TM} among healthy adults (n=20 [3 F and 17 M]; n=18 [0 F and 18 M]) with normal BMI (18.5-24.9 kg.m^{-2}) in supine position. This suggests that the performance of the Polar V800^{TM} in the detection of R-R intervals somewhat declined in adults with hypertension compared to healthy adults in supine position. The data acquisition and analyses methods were the same between our study and the studies of Giles et al., (2016) and Giles et al., (2017). However, the autonomic modulation of our subjects were substantially lower than those reported in both studies as evidenced by declined time domain measures (SDNN: 55.5 versus 61.4 [Giles et al., 2016] and 81.3 ms [Giles et al., 2017]; RMSSD: 33.8 versus 55.9 and 75.9 ms; pNN50%: 13.6 % versus 29.1% and 38.9%), frequency domain measures (HF ms^2: 740.8 versus 1827.0 [Giles et al., 2016] and 3978.2 ms^2 [Giles et al., 2017]; LFnu: 67.3 versus 41.0 and 36.8; HFnu: 32.7 versus 59.0 and 63.1; LF/HF: 3.6 versus 1.0 and 0.8), and non-linear domain measures (SD1: 27.1 versus 45.0 [Giles et al., 2016]

and 53.7 [Giles et al., 2017; SD2: 72.7 versus 84.0 and 100.9). Dysregulation/dysfunction of the ANS, a potential underlying mechanism for hypertension results in diminished R-wave amplitude (Baron et al., 1980), an important factor that can compromise the ability of HR monitors to accurately detect the R-R intervals that are used to calculate HRV measures. Therefore, hypertension may indirectly explain the higher error rate (i.e., higher number of distorted R-R intervals) observed in the current study.

The most common type of error encountered in the Polar V800^{TM} uncorrected R-R intervals was T4 (Table 2), which is in accordance with the previous studies reporting the percent of T4 error varying between 60% and 90% of total number of errors (Gamelin et al., 2006; Giles et al,., 2016; Giles et al., 2017). The T4 may be the result of a decrease in R-wave amplitude due to a loss or decrease in connection between the chest strap and skin (Giles et al., 2016). The second most commonly reported artifact in the Polar V800^{TM} uncorrected R-R intervals was T5 with (Table 2). Gamelin et al., (2006) thought that additional contractions during a single systole lead to T5, causing HR monitors to misidentify the T-waves or/and P-waves as R-waves. Of note, the Polar V800^{TM} uncorrected T1 and T6a errors can be seen on the corrected Bland–Altman plots (Figure 15) as outliers, a similar finding reported by Giles et al., (2016). Nevertheless, these errors cannot be easily seen in the R-R interval time series as opposed to T2, T3, T4, T5, and T6b errors.

The MC was the most successful method of correction with the highest decrease in bias (0.69 to 0. 37 ms for the UN R-R and the MC), LoA ranges (-215.80 to 214.42 ms and -41.20 to 41.94 ms for the UN R-R and the MC), and the highest increase in ICC (0.79 to 1.00 for the UN R-R and the MC) compared to the AC (bias: 3.79 ms, LoA ranges: -130.32 to 137.90 ms, and ICC: 0.91) and TBC (bias: 1.16 ms, LoA ranges: -92.67 to 94.98 ms, and ICC: 0.95) methods. The bias of the MC (0.37 ms) in this study, though somewhat larger, was consistent with Giles et al., (2016)

who reported bias of 0.06 between the Polar V800^{TM} and ECG. However, LoA ranges of the MC (-41.20 to 41.94 ms) we found were considerably wider than those (-4.33 to +4.45 ms) reported by Giles et al (2016). Further, while the bias of Polar V800^{TM} R-R intervals corrected by the MC was slightly lower than that of Polar S810^{TM} R-R intervals corrected by the MC (0.90), the LoA ranges we found (-41.20 to 41.94 ms) were noticeably higher than those (-11.0 to +13.0 ms) reported by Gamelin et al., (2006). These results are consistent with the higher error rate in the total number of R-R intervals of the current study (0.85%) than those of reported by Giles et al (2016) (0.08%) and Gamelin et al., (2006) (0.40%), indicating that quality of R-R intervals may impact the precision of the MC method in correcting artifacts.

The MC may take significant amount of time for artifact correction, and therefore, software packages like Kubios HRV offers automatic correction methods including the TBC and AC, which has been only available since 2017 with Kubios HRV Premium (ver 3.0). Previously, Giles et al., (2017) showed that the TBC of Kubios HRV (ver 2.2) did not properly correct the artifacts even though the software accurately identified the artifacts. Specifically, TBC of Kubios HRV (ver 2.2) replaced T4 error containing two missed beats with one interval rather than the required two. The same issue with T4 correction is still present with Kubios HRV Premium (ver 3.2). We observed that the AC, which was not available with Kubios HRV (ver 2.2) properly corrected the T4 errors containing two missed beats, the erroneous beat was replaced with the required two intervals. However, the AC was not able properly correct T4 errors containing >2 missed beats, the erroneous beat was replaced with one interval rather than the required >2 intervals. The problems in both AC and TBC in correcting T4 errors may explain why both methods resulted in larger biases and ESs, wider LoA ranges, and lower ICCs than those of the MC and ECG in the current study.

In addition to the issues with T4 error correction, the AC appears to always overcorrect three intervals at the beginning and the end of a sample regardless of the artifact condition. This may explain why the TBC performed better in correcting artifacts with smaller bias (1.16 versus 3.79 ms), tighter LoA ranges (-92.67 to 94.98 versus -130.32 to 137.90 ms), and higher ICC (0.95 versus 0.9) than the AC when both methods were compared. Nevertheless, the TBC of Kubios HRV Premium (ver 3.2) produced higher bias (1.6 versus 0.1 ms) and wider LoA ranges (-92.67 to 94.98 versus -0.15 to +0.24 ms) among adults with hypertension than the TBC of Kubios HRV (ver 2.2) among healthy individuals (Giles et al 2017). Since automatic correction methods in general perform better among healthy individuals with small number of artifacts (Peltola et al., 2012) higher error rate in the current study may explain the differences in R-R interval results.

Moreover, time, frequency, and non-linear HRV measures calculated from the Polar V800^{TM} R-R intervals corrected by the MC and those calculated from ECG showed excellent agreeability and interchangeability with consistent small biases, tight LoA ranges, trivial ES (i.e., ES <0.2, (Cohen et al., 2013), and excellent ICCs (i.e., ICC>0.90, Koo and Li, 2016), similar to levels of agreeability and interchangeability found in previous studies with the Polar V800^{TM} Giles et al (2016) and Polar S810^{TM} Gamelin et al., (2006). When time domain HRV measures (SDNN, RMSSD, and pNN50%) derived from the Polar V800^{TM} and ECG were compared, excellent agreement was found with small biases, tight LoAs, ICCs in all cases 1.00 and trivial ESs. The biases of the MC SDNN, RMSSD, and pNN50% (i.e., measures calculated from the Polar V800^{TM} R-R intervals corrected by the MC) were all < 1 ms, consistent with those reported by Giles et al., (2016) and Gamelin et al., (2006). The LoA ranges obtained for the MC SDNN (-0.70 to +1.12 ms), MC RMSSD (-1.82 to +2.18 ms), and MC pNN50% (-1.52 to +1.30 ms) were slightly higher than those reported by Giles et al., (2016) [(SDNN: -0.22 to +0.24 ms), (RMSSD: -0.32 to +0.32

ms), and (pNN50%: -1.20 to +0.70 %)] and Gamelin et al., (2006) [(SDNN: -0.47 to +0.63), (RMSSD: -1.17 to +1.58 ms), and (pNN50%:-2.47 to 3.04%)]. The ESs and ICCs of the MC SDNN, RMSSD, and pNN50% in the current study, Giles et al., (2016), and Gamelin et al., (2006) were trivial and excellent, respectively.

The frequency domain measures ($LFms^2$, LFnu, HFm^2, HFnu, and LF/HF) calculated from Polar V800^{TM} R-R intervals corrected by the MC displayed good agreement when compared with the corresponding ECG HRV measures. The biases of the MC $LFms^2$ (6.85 ms^2) and $HFms^2$ (9.94 ms^2) in the current study were higher than those reported by Giles et al., (2016) [($LFms^2$: -0.95 ms^2) and ($HFms^2$: 0.45 ms^2)] and Gamelin et al., (2006) [($LFms^2$: 0.06 ms^2) and ($HFms^2$: 0.39 ms^2)]. Nonetheless, the ICCs between the Polar V800^{TM} and ECG were excellent and the ESs were trivial ES (ES of $LFms^2$ < 0.005; ES of $HFms^2$) for both $LFms^2$ and $HFms^2$. On the other hand, biases of LFnu, HFnu, and LF/HF ratio were all <0.50, similar findings to those reported by Giles et al (2016) and Gamelin et al (2006). The LoA ranges obtained for the MC LF ms^2 (-65.77 to +79.48 ms^2), MC HF ms^2 (-44.20 to +64.07 ms^2), MC LFnu (-1.68 to 0.98), MC HFnu (-0.98 to +1.68), and LF/HF (-0.23 to +0.14) were wider than those reported by Giles et al (2016) [$LFms^2$: -6.25 ms2 +4.36), (HF ms^2: -27.95 to 28.84), LFnu: (-0.72 +0.56), HFnu: (-0.57 to +0.72), and LF/HF (-0.43 to +0.35)] and Gamelin et al., (2006) [$LFms^2$:-5.82 +5.94) ,($HFms^2$: -8.63 +9.42), and LF/HF: -0.18 +0.13)]. However, LoA ranges obtained for MC LFnu (-1.68 to 0.98) and MC HFnu (-0.98 to +1.68) were tighter than those reported by Gamelin et al., (2006) [LFnu:-1.90 +1.96) and (HFnu: -1.96 + 1.90). The ESs and ICCs of the MC LF ms^2, HF ms^2, LFnu, HFnu, and LF/HF in the current study, Giles et al., (2016), and Gamelin et al., (2006) were all trivial and excellent, respectively. However, when the MC was compared to the TBC and AC, the biases of

LFms^2, LFnu, HF ms^2, and HFnu were larger in the MC. Nonetheless, the trivial ESs and excellent ICCs were present between these variables of Polar V800^{TM} and ECG.

The non-linear measures (SD1, SD2, and SD2/SD1) calculated from Polar V800^{TM} R-R intervals corrected by the MC also displayed excellent agreement when compared with ECG. The biases of MC SD1 and SD2 were <0.50, similar findings to those reported by Giles et al., (2016) and Gamelin et al., (2006) who did not report SD2/SD1 ratio. The bias (0.01) and LoA range (-0.14 to +0.15) of SD2/SD1 ratio in the current study showed that SD2/SD1 is highly agreeable with that of ECG. The LoA range for SD1 (-1.29 +1.54) and SD2 (-0.55 +0.97) were slightly higher than those reported by Giles et al (2016) [(SD1: -0.21 +0.23) and (SD2: -0.20 +0.24)] and Gamelin et al 2006 [(SD1:-0.85 +1.15) and (SD2: -0.56 +0.60)].

With the exception of LFms^2 and HFm^2, the biases (<1 ms), ESs (<0.2), and ICCs (>0.96) of the all HRV measures calculated from the Polar V800^{TM} R-R intervals corrected by the TBC of Kubios HRV Premium (ver3.2) and those calculated from the Polar V800^{TM} R-R intervals corrected by the TBC (only option) of Kubios HRV (ver2.2) in Giles et al (2017) were highly consistent. However, LoA ranges for the TBC SDNN (-10.59 to 9.49 ms), the TBC RMSSD (-9.57 to 10.63 ms), and the pNN50% (-1.33 to 1.02%) were noticeably wider than those reported by Giles et al (2017) [(SDNN: -0.25 +0.24), RMSSD: (-0.69 +0.51), and pNN50% (-0.64 +0.72)]. Additionally, the LoA ranges for TBC LF ms^2 (-275.86 to 287.59), TBC HF ms^2 (-460.59 to 534.74), TBC LFnu (-4.04 to 4.01), TBC HFnu (-4.01 to 4.04), and LF/HF (-0.79 to 1.02) were substantially wider than those reported by Giles et al., (2017) [(LF ms^2: -15.34 to 12.2 ms^2), HF ms^2: (-36.11 to 29.077 ms^2), LFnu: (-0.82 to 0.672), HFnu: (-0.67 to 0.821), and LF/HF: (-0.23 to 0.18)]. Moreover, the LoA ranges obtained for TBC SD1 (-6.78 to 7.53) and TBC SD2 (-14.39 to

12.21) were also markedly wider than those reported by Giles et al (2017) [(SD1: -0.49 to 0.36 ms) and (SD2: -0.19 to 0.17 ms)].

The biases of AC SDNN (-1.60 ms), AC RMSSD (2.13), AC pNN50% (0.51%), AC LF nu (0.57), AC HF nu (-0.57), AC LF/HF (0.12), AC SD1 (1.51), AC SD2 (-2.97), and AC SD2/SD1 (-0.07) were larger than those of the TBC with the exception of AC LF ms^2 (0.80 ms^2) and AC HF ms^2 (-8.27 ms^2). In addition, the LoA ranges obtained for AC SDNN (-23.27 to 20.06), AC RMSSD (-25.57 to 29.84), AC pNN50% (-7.28 to 8.29), AC LF ms^2 (-369.45 to 371.06), AC HF ms^2 (-1115.84 to 1099.293), AC LF nu (-24.29 to 25.44), AC HF nu (-25.44 to 24.29), AC LF/HF (-1.76 to 2.00), AC SD1 (-18.12 to 21.14), AC SD2 (-26.11 to 20.17), and AC SD2/SD1 (-1.01 to 0.87) were substantially wider than those of the TBC method in the current study. Overall, both methods of Kubios HRV Premium (ver 3.2) had many of HRV measures outside of the LoA ranges, suggesting that individual HRV measures are not valid while group HRV measures could be acceptable with small biases, trivial ESs, and moderate to excellent ICCs.

5.1. Conclusion

This is the first reporting that validated the Polar V800^{TM} HR monitor in detecting R-R intervals and producing agreeable and interchangeable HRV measures in clinical populations, specifically in adults with hypertension. Of note, however, researchers should edit raw Polar V800^{TM} R-R intervals with an appropriate correction method to decrease bias and LoA ranges and thus to obtain valid HRV measures. Hypertension may be responsible for higher error rate, biases and wider LoA ranges in R-R intervals and HRV measures in the current study compared to those reported in healthy individuals since compromised autonomic function reduces R-wave amplitude, making the detection of R-R intervals difficult for Polar V800^{TM} HR monitor. Therefore,

correction of R-R intervals with artifacts among adults with hypertension is of higher importance than that of healthy individuals.

We recommend researchers to choose the MC as a first option for correcting Polar V800^{TM} artifacts because of the tightest LoA ranges, smallest ESs, and excellent ICCs of 1 for all HRV measures observed with the MC compared to the Kubios HRV Premium (ver 3.2) methods. Researchers can identify and correct the artifacts following the current guidelines (i.e., the MC) detailed in chapter 2 without the simultaneously recorded ECG. However, Kubios HRV Premium (ver 3.2) can be a second option for those who may find the MC complicated and laborious. In that case, the TBC should be preferred over the AC as the TBC produced smaller biases than the AC for all HRV measures with the exception of LF ms^2 and HFms^2 as well as tighter LoAs, and smaller ESs for all HRV measures. Since both automatic correction methods produced invalid individual HRV measures as substantial number of values were outside of LoA ranges researchers should only report group HRV measures when using Kubios HRV Premium (ver 3.2) for artifact correction. In addition, the previously described issues with the TBC and AC correction methods should be addressed in the next versions of Kubios HRV software packages in order to improve the data accuracy of HRV measures derived from Polar V800^{TM} in adults with hypertension.

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